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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Application of: | Barbara Ensoli | Confirmation No.: | 9400 |
| Application No.: | 09/555,534 | Art Unit: | 1648 |
| Filed: | May 31, 2000 | Examiner: | Humphrey, Louise Wang Zhiying |
| For: | HIV TAT, OR DERIVATIVES THEREOF FOR PROPHYLACTIC AND THERAPEUTIC VACCINATION | Attorney Docket No.: | 11340-003-999 |

**SECOND DECLARATION OF BARBARA ENSOLI, M.D., Ph.D.**
**UNDER 37 C.F.R. § 1.132**

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, BARBARA ENSOLI, M.D., Ph.D., do declare as follows:

1. I am the inventor of application No. 09/555,534 (hereinafter "the '534 application"). My education and experience are summarized on my Curriculum Vitae, which is attached hereto as Exhibit 1.

2. I designed and coordinated a Phase I human clinical trial using a vaccine comprising a recombinant, biologically active Tat protein (hereinafter "the Tat protein"), which had been expressed in *E. coli* and purified by ion-exchange chromatography and heparin-affinity chromatography, and formulated in human albumin and sucrose for use in humans according to good manufacturing practices (GMP), as required by national and international Regulatory Agencies, was used in the clinical trial.

3. The results of the clinical trial were summarized in a press release (attached hereto as Exhibit 2) dated July 5, 2005 by the Istituto Superiore di Sanità, which is the Assignee of the '534 application, and are described in more detail below.

## STUDY DESIGN

4. The trial was conducted in four clinical centers in Italy [San Raffaele Hospital (Milan), San Gallicano Hospital (Rome), Policlinico Umberto I (Rome), Spallanzani Hospital (Rome)] in healthy HIV-uninfected adult volunteers at low risk of infection (preventive protocol) and in HIV-1 infected asymptomatic adult volunteers naive to therapy (*i.e.*, having $CD4^+$ T cell counts $\geq 400/\mu l$, and viral load $\leq 50{,}000$ copies/ml) (therapeutic protocol).

5. Both studies were randomized, placebo-controlled, and double-blinded. Volunteers were randomized to one of two treatment arms with different routes of administration and blinded to dosage group. As indicated in Table A below, in Arm A, one group of volunteers received by subcutaneous (sc) administration the Tat protein with the Alum adjuvant at the dose of 7.5, 15 or 30 μg, at weeks 0, 4, 8, 12, 16; and another group of volunteers received Alum plus saline solution as placebo. In arm B, one group of volunteers received by intradermal (id) administration the Tat protein without adjuvant at the dose of 7.5, 15 or 30 μg, at weeks 0, 4, 8, 12, 16; and another group of volunteers received saline solution as placebo.

**TABLE A. STUDY POPULATION**

| | |
|---|---|
| For the preventive protocol, 18 volunteers were treated as follows: | |
| Arm A: Tat+Alum, sc | 7.5 μg: 3 subjects<br>15 μg: 3 subjects<br>30 μg: 2 subjects<br>placebo: 1 subject |
| Arm B: Tat, id | 7.5 μg: 2 subjects<br>15 μg: 2 subjects<br>30 μg: 2 subjects<br>placebo: 3 subject |
| For the therapeutic protocol, 25 volunteers were treated as follows: | |
| Arm A: Tat+Alum, sc | 7.5 μg: 3 subjects<br>15 μg: 2 subjects<br>30 μg: 4 subjects<br>placebo: 4 subjects |
| Arm B: Tat, id | 7.5 μg: 4 subjects<br>15 μg: 2 subjects<br>30 μg: 3 subjects<br>placebo: 3 subjects |

**SAFETY RESULTS**

6. Safety was evaluated by monitoring the volunteers for local and systemic adverse reactions during the course of the trial. Clinical evaluation of safety also included monitoring of haematological, biochemical (including liver and kidney functional parameters and coagulation assessment) and immunological parameters. Adverse events were continuously monitored by an independent "Adverse Events Monitoring Committee."

7. In both protocols, no clinically significant alterations were observed in all the haematological, biochemical and immunological parameters tested. Mostly mild (grade 1) local (pain, erythema, infiltrates at the site of injection), and/or systemic (fever, gastrointestinal symptoms, fatigue) adverse events were reported in the vaccinated subjects and no association with the vaccine dosage was observed.

8. No Severe Adverse Events (SAE) were reported in vaccinated subjects in the preventive protocol.

9. Two SAE were reported in the therapeutic protocol. One event was described as a transient paralysis of the left VII nerve. It occurred 22 days after the second immunization (Arm A, Tat 15 μg) and was completely resolved at clinical examination within the following 2 weeks. The event was discussed by the experts of the "Adverse Events Monitoring Committee." On the basis of the temporal interval with respect to the immunization, and the observation that facial paralysis has been reported to occur more frequently in HIV patients, the association to the immunization was defined as "possible." No request for unblinding was submitted by the investigator.

10. The second event was described as lipothymia-nausea. The event occurred 50 minutes after the second immunization (Arm A, Tat 7.5 μg). The episode lasted for 10 minutes only, and then resolved without residual effects. The event was reported as "serious" because the clinical investigator directed the volunteer to the Hospital Emergency. However, the volunteer decided not to comply with the investigator suggestion. The relationship to study drug was defined as "possible" in the SAE form. As a possible concomitant/alternative cause of the event, the investigators refer to "possible gastric block after breakfast." Indeed, 30 minutes after the immunization (and then 20 minutes before the occurrence of the event),

and in contrast with the guidelines provided by investigator, the patient had breakfast. No request for unblinding was submitted by the investigator.

**IMMUNE RESPONSE TO THE VACCINE**

11. Immunogenicity was evaluated by assessing the induction of anti-Tat humoral and cellular immune responses.

**Humoral Immune Response**

12. Assessment of anti-Tat humoral immune response was performed by the detection and titration of specific anti-Tat antibodies in sera from HIV-1 negative (preventive protocol) and HIV-1 positive (therapeutic protocol) individuals. In particular, the presence and titer of specific anti-Tat IgM, IgG and IgA were evaluated at week 0 (baseline), at week 8 (28 days after the II immunization), at week 12 (28 days after the III immunization), at week 16 (28 days after the IV immunization), and at week 24 (56 after the V immunization).

**Humoral Immune Response – Preventive Protocol**

13. As shown in Table 1 (attached hereto as Exhibit 3), specific anti-Tat IgM and IgG were induced by all vaccine doses in both arms in all the vaccinated subjects [8/8 (100%) in Arm A; 6/6 (100%) in Arm B], while specific anti-Tat IgA were induced in 12/14 (86%) of the immunized volunteers [8/8 (100%) in Arm A; 4/6 (67%) in Arm B]. No specific anti-Tat antibodies were detected in sera from individuals randomized in the placebo groups [0/1 (0%) in Arm A; 0/3 (0%) in Arm B]. As shown in Figure 1 (attached hereto as Exhibit 4), higher level of specific anti-Tat IgG and IgA were induced by the sc administration of 7.5 μg of Tat+Alum (Arm A); while higher levels of specific anti-Tat IgM were induced by the id administration of 7.5 μg Tat alone (Arm B). As shown in Figure 2 (attached hereto as Exhibit 5), the epitope mapping performed on the anti-Tat positive sera (for both IgM and IgG) showed a broad recognition of linear epitopes, covering the acidic, cysteine-rich, basic, glutamine and/or RGD regions of the Tat protein in all subjects. The broadest responses were observed at 7.5 μg of Tat and were maximal for IgM by the id route, and for IgG by the sc route of administration.

14. The anti-Tat neutralizing activity of sera from subjects vaccinated with the Tat protein was evaluated by the "rescue assay." This assay is based on HLM1 cells obtained

from a HeLa-CD4+ cell line containing an integrated copy of a HIV-1 Tat-defective provirus. Replication of this virus can be rescued by the addition of exogenous Tat. The neutralizing activity is measured by the ability of sera to inhibit the replication of the defective provirus induce by exogenous Tat by a p24 antigen capture assay. Tests were performed with sera from all the HIV-1 negative individuals vaccinated subcutaneously or intradermally, and anti-Tat neutralizing titers evaluated. As shown in Figure 3 (attached hereto as Exhibit 6), all volunteers displayed a specific anti-Tat neutralizing activity, whereas no neutralizing activity was observed with sera from the same individuals before immunization (baseline). As shown in Figure 4 (attached hereto as Exhibit 7), the statistical analysis of the data indicated a significant correlation of the neutralizing activity with the specific anti-Tat IgM ($p=0.0039$) and IgG ($p=0.0541$) responses.

**Humoral Immune Response – Therapeutic Protocol**

15. As shown in Table 2 (attached hereto as Exhibit 8), specific anti-Tat IgM were present in 3/18 (17%) subjects at baseline [3/9 (33%) in Arm A; 0/9 (0%) in Arm B] as a response to the natural infection and, after vaccination, were induced in 15/18 (83%) of the immunized individuals [7/9 (78%) in Arm A; 8/9 (88%) in Arm B]. One out of seven (14%) subjects was positive at baseline in the placebo group; a transient induction (at 1 time point) of specific anti-Tat IgM was reported in one volunteer randomized in the placebo group (Arm B). Anti-Tat IgG were present in 2/18 (11%) subjects at baseline [1/9 (11%) in Arm A; 1/9 (11%) in Arm B] as a response to the natural infection and, after vaccination, were induced in all immunized individuals [18/18 (100%)]. One out of seven (14%) subjects was positive at baseline in the placebo group; no induction of specific anti-Tat IgG was reported in this group. Anti-Tat IgA were present in 0/18 (0%) subjects at baseline [0/9 (100%) in Arm A; 0/0 (100%) in Arm B] and, after vaccination, were induced in 11/18 (61%) of the immunized individuals [6/9 (67%) in Arm A; 5/9 (55%) in Arm B]. One out of seven (14%) subjects was positive at baseline in the placebo group; no induction of specific anti-Tat IgA was reported in this group.

16. As shown in Figures 5 and 6 (attached hereto as Exhibits 9 and 10, respectively), higher level of specific anti-Tat IgM, IgG and IgA were induced by the sc administration of Tat+Alum; higher levels of specific anti-Tat IgM and IgA were induced by 7.5 μg of Tat; and higher levels of specific anti-Tat IgG were induced at all dosages. As

shown in Figure 7 (attached hereto as Exhibit 11), the epitope mapping performed on the anti-Tat positive sera (for both IgM and IgG) showed a broad recognition of linear epitopes, covering the acidic, basic, glutamine and/or RGD regions of the Tat protein after immunization. As shown in Figure 8 (attached hereto as Exhibit 12), no recognition of Tat-specific linear epitopes was observed within the placebo group.

17. The anti-Tat neutralizing activity of sera from subjects before (baseline) and after immunization were evaluated by the "rescue assay" described above. As shown in Figure 9 (attached hereto as Exhibit 13), all sera displayed different levels of neutralizing activity at baseline, however, an increase of this activity was observed in all subjects not reaching a plateau at baseline. As shown in Figure 10 (attached hereto as Exhibit 14), the induction of the neutralizing activity (defined as a $\Delta$ of neutralization versus baseline), as well as the induction of specific anti-Tat IgM and IgG (defined as a ratio versus baseline), were statistically significant in the vaccinated volunteers, and were also significantly different from that observed in the placebo group.

**Cellular Immune Response**

18. Assessment of anti-Tat cellular immune response was performed by the evaluation of *in vitro* IFN-$\gamma$ production in response to Tat (by Elispot assay), *in vitro* IL-4 production in response to Tat (by Elispot assay), and lymphoproliferative response to Tat (by 3H-thymidine incorporation). These tests were performed with peripheral blood mononuclear cells (PBMC) obtained from HIV-1 negative (preventive protocol) and HIV-1 positive (therapeutic protocol) individuals. In particular, the presence of a specific anti-Tat cellular immune response was evaluated at week 0 (baseline), at week 4 (7 days after the II immunization), at week 12 (7 days after the IV immunization), and at week 16 (7 days after the V immunization).

**Cellular Immune Response – Preventive Protocol**

19. As shown in Table 3 (attached hereto as Exhibit 15), a specific anti-Tat cellular immune response was induced in 13/14 (93%) vaccinated individuals [7/8 (87%) in Arm A; 6/6 (100%) in Arm B]. In particular, proliferation activity to Tat was induced in 9/14 (64%) vaccinated individuals [5/8 (62.5%) in Arm A; 4/6 (67%) in Arm B]. IFN-$\gamma$ production in response to Tat was induced after immunization in 5/14 (36%) individuals [2/8

(25%) in Arm A; 3/6 (50%) in Arm B]. IL-4 production in response to Tat was induced after immunization in 12/14 (86%) individuals [7/8 (87.5%) in Arm A; 5/6 (83%) in Arm B]. No anti-Tat cellular immune response was observed in the individuals randomized in the placebo groups. As shown in Figure 11 (attached hereto as Exhibit 16), the frequency of responders and the intensity of the immune responses were comparable for proliferation and IL-4 Elispot, however, a higher percentage of positive response for the IFN-γ Elispot were observed with the id immunization (Arm B).

**Cellular Immune Response – Therapeutic Protocol**

20. As shown in Table 4 (attached hereto as Exhibit 17), a specific anti-Tat cellular immune response was present at baseline as response to the natural infection in 15/18 (83%) individuals randomized in the groups to be treated with the Tat vaccine [8/9 (89%) in Arm A; 7/9 (78%) in Arm B], and in 6/7 (86%) individuals randomized in the placebo groups [4/4 (100%) in Arm A; 2/3 (67%) in Arm B]. After immunization, specific anti-Tat cellular responses were observed in 18/18 (100%) individuals, while a decrease was observed in the placebo groups [4/7 (57%)]. In particular, proliferation activity to Tat was present at baseline in 11/18 (61%) individuals randomized in the groups to be treated with the Tat vaccine [6/9 (67%) in Arm A; 5/9 (55%) in Arm B], and in 2/7 (19%) individuals randomized in the placebo groups. After immunization, anti-Tat proliferation was observed in 15/18 (83%) individuals [7/9 (78%) in Arm A; 8/9 (89%) in Arm B], while a decrease was observed in the placebo groups [1/7 (14%)]. IFN-γ production in response to Tat was present, at baseline, in 10/18 (55%) individuals randomized in the groups to be treated with the Tat vaccine [4/9 (44%) in Arm A; 6/9 (67%) in Arm B], and in 5/7 (71%) individuals randomized in the placebo groups. After immunization, this response was observed in 14/18 (78%) individuals [6/9 (67%) in Arm A; 8/9 (89%) in Arm B], while a decrease was observed in the placebo groups [4/7 (57%)]. IL4 production in response to Tat was present, at baseline, in 3/18 (17%) individuals randomized in the groups to be treated with the Tat vaccine [1/9 (11%) in Arm A; 2/9 (22%) in Arm B], and in 1/7 (14%) individuals randomized in the placebo groups. After immunization, similarly to what observed for IFN-γ production, this response resulted increased in the immunized volunteers, with 7/18 (39%) positive individuals [2/9 (22%) in Arm A; 5/9 (55%) in Arm B]. All the individuals in the placebo groups were negative at the end of the study [0/7 (0%)].

21. As shown in Figure 12 (attached hereto as Exhibit 18), the frequency of responders and the intensity of the cellular immune responses were comparable, however, a higher percentage of positive responses for the proliferation Elispot and IFN-γ Elispot were observed with the id immunization (Arm B).

**CD4± T Cell Counts and HIV Plasmaviremia – Therapeutic Protocol**

22. The therapeutic trial allowed the assessment of the HIV viral load and $CD4^+$ T cell decline, which are the two key parameters used to evaluate HIV replication and immunodeficiency, respectively, and, therefore, disease progression.

23. In particular, HIV-RNA plasmaviremia and absolute $CD4^+$ T cell number were analyzed by comparing the values observed at several time points after treatment to the baseline values. Baseline was defined as the average of the values obtained in three determinations performed prior to treatment.

24. As shown in Figure 13 (attached hereto as Exhibit 19), concerning HIV plasmaviremia, the average ratio of the viremic levels after treatment versus baseline, was as follows: in both arms the ratio was significantly above the baseline (=1) in the placebo group, whereas it was below baseline in vaccines (cumulative data from the three vaccine doses). The cumulative data from both arms showed the same results. These data indicate that vaccination with the Tat protein was associated with the control of HIV replication.

25. As shown in Figure 14 (attached hereto as Exhibit 20), concerning the $CD4^+$ T cell counts, the average change (Δ) after treatment versus baseline was as follows: in both arms, only the placebo group showed significant declines of $CD4^+$ T cell counts below the baseline levels. In contrast, the vaccinated subjects not only failed to show that $CD4^+$ T cell count declines below baseline, but had more stable $CD4^+$ T cell counts that were above the baseline levels. The cumulative data from both arms showed the same results. These data indicate that vaccination with the Tat protein was associated with stabilization of $CD4^+$ T cell numbers.

**CONCLUSION**

26. Altogether, the data indicate that the Tat vaccine was safe in both uninfected and infected individuals. In addition, the Tat vaccine was very efficient at inducing both

specific humoral and cellular immune responses against the Tat protein, including the induction of neutralizing antibodies against Tat. Further, this immune response was able to control HIV replication in infected volunteers, thus preventing $CD4^+$ T cell decline which is the most relevant parameter of disease progression.

27. In my judgment and opinion, the results, summarized in Paragraph 26, lead to the conclusion that the Tat vaccine has efficacy in the treatment of HIV infection. As a consequence, this vaccine should be capable of inducing protective effects also in HIV uninfected individuals.

28. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that I make these statements with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application, and any patent issuing thereon.

Date: April 27, 2007

Barbara Ensoli

Barbara Ensoli, M.D., Ph.D.

# CURRICULUM VITAE

Name: Dr. Barbara Ensoli

Date and Place of Birth: January 23, 1960; Latina, Italy

**Education:**

| | | |
|---|---|---|
| 1978-1984 | - | University of Rome "La Sapienza", Medical School, Rome, Italy |
| 1984 | - | M.D., University of Rome "La Sapienza", Rome, Italy |
| 1984-1987 | - | Residency in Allergy and Immunology, University of Rome "La Sapienza," Italy |
| 1987-1991 | - | Ph.D. (Immunology), University of Rome, Italy |

**Other Training:**

| | | |
|---|---|---|
| 1986 | - | Authorised User for Radioactive Compounds, Radiation safety branch, National Institute of Health (NIH), Bethesda, Maryland, USA. |
| 1988 | - | Molecular Biology course "Special topics in recombinant DNA technology", Biotrac, Foundation for Advanced Education in the Science, NIH, Bethesda, Maryland, USA. |
| 1989 | - | Biochemistry's course of "Separation techniques", Biotrac, Foundation for Advanced Education in the Science, NIH, Bethesda, Maryland, USA. |
| 1993 | - | Responsible Supervisor for Radioactive compounds, National Safety Branch, National Institute of Health (NIH), Bethesda, Maryland, USA. |

**Brief Chronology of Employment:**

| | | |
|---|---|---|
| 1982-1986 | - | Internship, Department of Allergy and Clinical Immunology, Laboratory of Immunology, University of Rome "La Sapienza," Italy |
| 1986 | - | Fellowship, Italian Cancer Society, Laboratory of Tumor Cell Biology (LTCB), National Cancer Institute (NCI), NIH, Bethesda, Maryland, USA |
| 1986-1990 | - | Visiting Fellow, LTCB, NCI, NIH, Bethesda, Maryland, USA |
| 1990-1993 | - | Visiting Associate, LTCB, NCI, NIH, Bethesda, Maryland, USA |
| 1993-1994 | - | Visiting Scientist, LTCB, NCI, NIH, Bethesda, Maryland, USA |
| 1994-1996 | - | Visiting Scientist (Tenure Track), Head of the Vascular Biology and AIDS-associated Malignancies Unit, LTCB, NCI, NIH, Bethesda, Maryland, USA |
| 1996-1999 | - | Director of Research, Laboratory of Virology, Istituto Superiore di Sanità, Rome, Italy |
| 2000-2004 | - | Director, Retrovirus Division, Laboratory of Virology, Istituto Superiore di Sanità, Rome, Italy |
| 2004-2005 | - | Director, AIDS Division, Department of Infectious, Parasitic and Immune-mediated Diseases, Istituto Superiore di Sanità, Rome, Italy. |
| 2005-Date | - | Director, National AIDS Center, Istituto Superiore di Sanità, Rome, Italy. |

**Description of the National AIDS Center**

Mission of the National AIDS Center is to develop and to clinically test preventive and therapeutic vaccines as well as new interventions against HIV/AIDS and related syndromes based on pathogenesis and correlates of protection.
The Center is organized in 5 *Divisions,* each composed of *Units,* centered on the key activities required by the translational research program of the Center.

Divisions and Units:

*1. Pathogenesis of Retroviruses*
Molecular Retrovirology
Applied Retrovirology

The Division of Pathogenesis of Retroviruses is mainly involved in the following issues: i) the study of the role of macrophages in the cell-to-cell HIV spread. These cells have been found transmitting HIV products to epithelial, neuronal, or dendritic cells. The study of the functional consequences of the cell-to-cell transmission of single cell products is currently under investigation; ii) the analysis of the contribution of HIV-1 Nef to the AIDS pathogenesis, in particular referred to its effects on macrophages, and; iii) the development of innovative vaccine approaches based on the use of either HIV or MLV-based Virus-Like Particles (VLPs) for the antigen delivery . Pre-clinical studies for the VLP-mediated induction of immunity against viruses (HIV, HPV, HCV) or tumors (e.g., melanoma) are ongoing.

*2. Virus-Host Interaction (Immunology Core-Lab)*
Humoral Immunity
Innate and Adaptive Cellular Immunity
Immune and Vascular System Cross-Talk

The main tasks of the Division are: i) Discovery: development/implementation of new-generation immunogens and vaccination strategies through the study of virus-host interaction; ii) Translation (perspective): evaluate immunogenicity and correlates of protection in animal models for selection of candidates for human studies; iii) Scientific support: immunological assays, end-points and immunologic read-out of basic and preclinical studies of the Center; iv) Organization: integration of the activities of the Units and cross-talk with the other Divisions, Services and Secretariat; v) Management/Administrative: resources and personnel management (according to Center guide-lines).

*3. Experimental Retrovirology and Non-Human Primate Models (Virology Core Lab)*
Viral Infectivity
Viral Tropism
Non-Human Primate Models

The main tasks of the Division are: i) studies on the pathogenesis of the viral and retroviral infections (SIV, HIV, EBV, Type D retrovirus) in preclinical models, ii) development of new vaccine approaches and of delivery systems, iii) conduction of vaccine preclinical trials (safety, immunogenicity and efficacy) in non human primate model, iv) production and in vivo titration of lentivirus (SIV) and chimeric SIV/HIV (SHIV) viruses and v) generation of new chimeric SHIVs. The Division, as a core-lab of virology, develops and validates biological (virus isolation),

molecular (quantitative RT-, DNA-PCR assay) and immunohistochemical methodologies to detect viruses in tissues and in biological samples. The Division participates to international working groups in order to implement biological and molecular methodologies. To accomplish these tasks, the Division is organized in unit facing themes of the basic research (infectivity and viral tropism) and of translational research by using non human primate model as a tool for the development of preventative and therapeutic vaccine approaches for clinical trials.

*4. Clinical Trials (Immunology and virology Core-Lab)*

Core Lab ISS/IFO Joint Unit
Clinical Trial Management
Data Analysis and Validation
Preclinical Validation

Main activities: Core Laboratory of Immunology and Virology (development, standardisation and validation of specific assays, SOPs preparation, proficiency testing); Coordination of vaccine production related activities (production, formulation, testing, labelling, packaging, distribution, dossier preparation and submission); Clinical Trial Management (connections with Competent Authorities, coordination of clinical sites activities, preparation of relevant clinical documentation, coordination of CRO activities, preparation of safety report, interim report, final clinical study report); Data Analysis and Validation (data management, database design and testing, statistical analysis plan design, statistical analysis); Preclinical Validation (preclinical safety evaluation of vaccine candidates in small animals, single and repeated dose toxicity studies, preclinical immunogenicity evaluation).

*5. Retrovirus Infections in Developing Countries*

Epidemiology and Humoral Immunity
Pathogenesis and Cell-Mediated Immunity

The Division of Retrovirus Infections in Developing Countries of the National AIDS Center has been created under the need to transfer to clinical practice the knowledge and products of the basic research on HIV/AIDS. Main activities of the Division are the strengthening of laboratory and clinical capacity for future advanced HIV vaccine clinical testing and transfer of technology and training of local medical doctors, scientists and technicians in order to favourite the growth of biomedical knowledge, not only in the HIV/AIDS field. To these aims, the Division is involved in these countries with epidemiological, immunological and virological studies and social-behavioural studies aimed at evaluating the perception of risk to be infected by HIV and willingness to participate to vaccine clinical trials. Strictly connected with the need to provide a picture of HIV/AIDS infection in Developing Countries, are the immunopathogenesis studies aimed at evaluating the natural history of HIV infection in these countries, often different from that described in developed countries for environmental, immunological and genetic reasons. Finally, the Division is the Italian reference for the control of the laboratory tests for HIV infection and is involved in activities of support to the Blood Transfusion Centre of the Policlinico Hospital, in Rome, for the diagnosis of HIV infection.

**National (intra and extramural) Program of the Center**

- National AIDS Program (Ministry of Health)
- ICAV (Italian Concerted Action on HIV/AIDS Vaccine development)
- New formulation and Delivery System Program (Ministry of Industry, University and Research)
- Vaccine Clinical Trials in Italy (Ministry of Health)

**EU Programs of the Center**

- AVIP (AIDS Vaccine Integrated Program)
- VIAV (Very Innovative AIDS Vaccine)
- MUVAPRED (Mucosal Vaccines for Poverty related Diseases)
- PINT (Pathogenesis Interaction Nef/Tat)
- NAV (Novel AIDS Vaccines)
- EAPN (European HIV/AIDS Preventive Network)

**International Programs of the Center**

- ISS/Chiron (Ministry of Health)
- Italy/USA (ISS/NIH)
- Capacity building and Tat vaccine clinical trials in Developing Countries (MAE)
- Global Vaccine Enterprise

**Program Coordinator:**

- National AIDS Program, Ministry of Health, Italy (2006)
- "AIDS Vaccine Integrated Projec" (AVIP), FP6, European Commission (2004-2009)
- "Very Innovative AIDS Vaccine" (VIAV), FP6, European Commission (2005-2008).
- Concerted Action on "The use of HIV protease inhibitors in AIDS-associated Kaposi's sarcoma", National AIDS Program, Ministry of Health, Italy.
- Concerted Action on HIV/AIDS vaccine development (ICAV), National AIDS Program, Ministry of Health, Italy (2003, 2004).
- Italy-USA inter-institute (Istituto Superiore di Sanità and NIH) cooperation on "HIV/AIDS vaccine development" (2000).
- "Joint Program ISS/Chiron (Novartis Vaccines&Diagnostics) for the development of a combined vaccine against HIV/AIDS". Chiron Corporation (Novartis Vaccines&Diagnostics), Emeryville, California, USA (2002-2005).
- Principal Investigator (for ISS), Mucosal Vaccines for Poverty Related Diseases (MUVAPRED) Integrated Project, FP6, European Commission (2003-2008).
- Principal Investigator (for ISS), Network of Exellence "European Vaccines and Microbicides Enterprise" (EUROPRISE), FP6, European Commission (2007-2012).
- "Intervention Framework for Comprehensive Response to HIV/AIDS Cross-border in Selected Development Regions of South Africa, Swaziland and Mozambique" (funded by Foreign Affairs'Ministry).

**Member of Committees and Societies:**

- National AIDS Program, Ministry of Health, Italy:
  - Member, Scientific Committee for the Project: "Epidemiologia e modelli di ricerca assistenziale", National AIDS Program, Ministry of Health, Italy (1999)
  - Member, Scientific Committee for the program coordination in fighting and preventing from HIV infection and correlated diseases(1999)
  - Member, Scientific Committee for the Project: "Patologia Clinica e Terapia dell'AIDS", National AIDS Program, Ministry of Health, Italy (1999)
  - Responsible for the Scientific Committee "Italy-USA for the development of vaccines against HIV/AIDS", National AIDS Program, Ministry of Health, Italy (2000).

- Responsible for the Scientific Committee "Vaccine AIDS Project", National Intramural AIDS Program, Ministry of Health, Italy (2000).
- Responsible for the Scientific Committee "Italy-USA for the development of vaccines against HIV/AIDS", Intervention and Surveillance Program, National AIDS Program, Ministry of Health, Italy (2000).
- Member, Scientific Committee for the Project: "Patologia Clinica e Terapia dell'AIDS", National AIDS Program, Ministry of Health, Italy (2001)
- Scientific Responsible, Concerted Action on HIV/AIDS vaccine development (ICAV), National AIDS Program, Ministry of Health, Italy (2003, 2004).
- Scientific Responsible, Project: "Eziopatogenesi e studi immunologici e virologici dell'HIV/AIDS", National AIDS Program, Ministry of Health, Italy (2004)

- Committee for patent activity of the Istituto Superiore di Sanità, Ministry of Health, Italy (1999)
- National Committee for AIDS, Ministry of Health, Italy (1999)
- Vice-President, National Committee for AIDS, Ministry of Health, Italy (2006-2008).
- VII International AIDS Conference Program Committee (Florence, June 1-21, 1991)
- European Conference on Experimental AIDS Research (ECEAR), Scientific Committee and Organizative Committee
- International Committee, 8th European Conference on Experimental AIDS Research (ECEAR) (May 26-28, 2006, Naples, Italy)
- AIDS-associated Malignancies Working Group (AMWG) Committee, NCI, NIH, USA
- Italian Association of Immunopharmacology, Scientific Committee
- National Committee for the "Challenge against AIDS and other infectious diseases", Ministry of Health, Italy
- Organizing Committee, 3rd International Conference on Human Herpesvirus-6, -7 and -8 (Tampa, Florida, May 13-15, 1999)
- Italian expert for the European Agency for the Evaluation of Medicinal Products (EMEA)
- European Molecular Biology Organization (EMBO) (2000)
- American Association of Immunologists (AAI)
- UNESCO delegate for Italy, Vice-President of the Natural Sciences Committee (2001)
- United Nations General Assembly for HIV/AIDS (New York , 25-27 June 2001), member of the delegation representing Italy
- Permanent Observatory, "Marisa Bellisario Foundation", for the analysis and the commentary of parliamentary initiatives in public health and scientific research
- Workgroup on Infrastructures and on excellent European networks in the field of biosafety and biotechnology instituted by the National Committee for Biosafety and Biotechnology, Presidenza del Consiglio dei Ministri (2002)
- Conference Organization, XIV International AIDS Conference, Barcelona (July 7-12, 2002).
- Member of the WHO-UNAIDS HIV Vaccine Advisory Committee (VAC) (2004-2005).
- Member of Guarantee Committee, Consiglio Nazionale delle Ricerche (CNR), Rome (2005).
- Member of Scientific Program Committee, AIDS Vaccine Conference, Amsterdam (August 30-September 1, 2006).
- Member of Scientific Committee, "Giuseppina Mai Foundation" to promote public and private research and interaction among universities, public institutes and industries for technology transfer activities (2006).

**Cooperations with Industries:**

Principal Investigator, "Therapy of AIDS-associated Kaposi's sarcoma and other AIDS-associated malignancies by using phosphorothioate oligonucleotides and other analogues directed against basic

fibroblast growth factor, other cytokines and the HIV-1 Tat protein". Lynx Therapeutics, Inc., Hayward, California, USA (1993-1995).

Principal Investigator, "Joint Program ISS/Chiron (Novartis Vaccines&Diagnostics) for the development of a combined vaccine against HIV/AIDS". Chiron Corporation (Novartis Vaccines&Diagnostics), Emeryville, California, USA (2002-2005).

Principal Investigator, Master Clinical Study Agreement for the use of the HIV protease inhibitor Indinavir in the treatment of tumors. Merck & Co., New Jersey, USA (2004).

Principal Investigator, Agreement for the production of vaccine clinical batches, Phase II trial for the development of a vaccine against HIV/AIDS. INJECTALIA Srl, Santa Palomba, Rome Italy (in collaboration with University of Urbino and AVITECH – Antigen Production Unit, Fano, Italy) (2007).

**Honors and Other Special Scientific Recognition:**

1984 - Summa cum laude (M.D. Thesis)
1987 - Summa cum laude (Residency)
1990 - "Italian Society for AIDS Research" award for the most significant publication in the field of AIDS research for 1989, "AIDS-Kaposi's sarcoma-derived cells express cytokines with autocrine and paracrine growth effects." Science 243: 223-226, 1989
1991 - Summa cum laude (Ph.D. Thesis)
1991 - Proposed for Tenure and Head of the Unit of Vascular Biology and AIDS-associated Malignancies by Site Visit Review Board, NCI, NIH, USA
1992 - Award from the NIH for contribution to technology transfer from the LTCB to the private sector
1993 - Award from the NIH for contribution to technology transfer from the LTCB to the private sector
1994 - Award from the NIH for contribution to technology transfer from the LTCB to the private sector
1994 - Grandfathered into Tenure Track Program, NIH, USA
1999 - "Italian Society for AIDS Research" award for the most significant publication in the field of AIDS research for 1999, "Control of SHIV-89.6P-infection of cynomolgus monkeys by HIV-1 Tat protein vaccine". Nature Medicine 5: 643-650, 1999
1999 - International Award "San Valentino d'Oro" for professional activity against AIDS.
1999 - International Award " Marisa Bellisario" for professional activities.
1999 - International Award "Inner Weel" for professional merits.
2000 - "Fiore di Roccia" Award for scientific achievements.
2000 - Honor for scientific achievements conferred by the President of the Italian Republic Mr. A. Ciampi.
2000 - "Eleonora Benvenuti Turziani" Award for the year 2000, Perugia, for scientific and professional merits.
2000 - "Universum" International Award for the year 2000, Potenza, for scientific and professional merits.
2000 - "Woman of the year 1999" Award conferred by the magazine "Grazia" (Mondadori Ed.) for scientific and professional merits.
2000 - Honorary Member of Medical Women's International Association (M.W.I.A).
2001 - Honor (Cavaliere dell'Ordine al Merito della Repubblica Italiana) conferred by the President of the Italian Republic Mr. A. Ciampi, 2001.

2001 - "Domenico Marotta" Award for year 2000, conferred from professional merits by the National Academy of Sciences (also known as "National Academy of XL") at the Royal Museum of Mineralogy, Naples.

2001 - "Graziella Fumagalli" Award for year 2001, conferred from professional merits by the Confartigianato Donna Impresa, Padova.

2002 - "Santa Caterina" Award for year 2002, conferred from professional merits by "S. Caterina d'Oro" and "Cateriniani nel Mondo", Siena.

2002 - "Graziella Fumagalli" Award for year 2002, conferred from professional merits by the Confartigianato Donna Impresa, Padova.

2002 - "Altipiani d'Arcinazzo"Award for year 2002, conferred from scientific and professional merits.

2002 - "R.O.S.A." Award for year 2002 conferred from professional merits by the Canova Club.

2003 - "Minerva" Award for the year 2003 conferred for scientific and professional merits.

2003 - "Italiani nel Mondo" Award for year 2003 (III Edition) conferred for professional merits by the Ministero per gli Italiani nel Mondo (Marzio Tremaglia Foundation), supported by President of the Italian Republic.

2004 - Honor (Ufficiale) conferred by the President of the Italian Republic Mr. A. Ciampi, 2004.

2004 - "Ordres des femmes de la jarretiere" award for the year 2004 for scientific and professional merits.

2004 - International Award "Paul Harris Fellow" conferred by the Rotary Foundation for professional activities.

2004 - "Vittorio Bachelet" award conferred for her Vaccine against AIDS.

2004 - "Gentile da Fabriano" national award conferred for scientific and professional merits.

2004 - "Stella" national award for the year 2004, conferred by the Associazione Italiana Ospedalità Privata (A.I.O.P.) for professional activities.

2005 - "Donna Emerita" national award conferred by the Federazione Italiana "Donne, Arti, Professioni ed Affari", Padova, for scientific and professional merits.

2005 - "Premio Internazionale alle Libertà", conferred by the Associazione di Cultura Liberale "Società Libera", Milano, for scientific and professional merits.

2005 - "Eminent Scientist of the Year 2005" International Award for Europe, conferred by the International Research Promotion Council – World Scientist Forum International Award –Science and Medicine, for scientific and professional merits.

2006 - "Simpatia" national award for the year 2006 (Sala della Protomoteca, Campidoglio, Rome) conferred for scientific and professional merits.

2006 - "Provincia Capitale" Award (Palazzo Valentini, Rome), conferred by On. Gasbarra, President of the Province, for scientific and professional merits.

2006 - "Foyer des Artistes" Award (Teatro Capranica, Rome), conferred by the "Centro Nazionale Arte, Cultura e Scienza", for scientific and professional merits.

**Teaching:**

- Georgetown University, Washington, USA; Tulane University School of Medicine, New Orleans, USA; NIH, Bethesda, Maryland, USA; Columbia University, New York, USA.

- External Examiner: MSc in Medical Sciences (Medical Virology), Candidate Mr. TS Scriba (12721255-1997), University Office Tygerberg Campus, Africa, 2002.

- Member of Teaching Scientific Committee of the International PhD courses, University of Ferrara, Stockholm and German Research Center For Biotechnology (Germany).

- Courses to Medical Doctors on AIDS pathogenesis, AIDS-associated tumors, angiogenesis, HIV/AIDS vaccine development at the University of Ferrara, Ferrara, Italy.

- Appointed Professor, Medical School, University of Ferrara, Ferrara, Italy (1999/2000, 2000/2001, 20001/2002, 2002/2003, 2003/2004, 2004/2005, 2005/2006, 2006/2007).

- Advisory of the Teaching Scientific Committee of the PhD courses in Experimental Oncology, University of Ferrara, Ferrara, Italy (2004).

- Teacher for Medical School (2005-2006), on "HIV infection and AIDS pathogenesis", title of the lecture: "immune-prevention and vaccination and HIV vaccination - new prevention and therapy routes", University of Padua, Padua.

- Teacher for and academic lecture: "anti-Tat HIV-1 vaccine: from basic science to clinical trials" at Biomedical Science Dept., General Pathology Section, University of Modena and Reggio Emilia.

- Member of the Microbiology and Virology Post-Graduate School Committee and University teacher for the integrative course "Innovative vaccines" (2003-2004, 2004-2005), Microbiology and Virology School, Faculty of Medicine, University of Brescia, Italy.

- University teacher on "Novelty in oncohaematology" (November 29-30, 2002, Legnano), Legnano Hospital, Italy.

- Member of Teaching Scientific Committee of the International PhD courses in Medical Immunemicrobiology, University of Rome "Tor Vergata", Rome, Italy.

- Warrant (designated by "Centro Nazionale Ricerche", year 2005) for the disciplinary aggregation of expertise, with the task of proposing the nominees of the members of the boards of examiners recruiting personnel (investigators and technicians) and for personnel promotions.

**Patents:**

- Ensoli, B. and Gallo, R.C.: Method for treating Kaposi's sarcoma with antisense oligonucleotides, (U.S. Patent Application, No. 08/072,575, priority date June 4, 1993; PCT May 17, 1995, No. PCT/US94/05467, Publication No. WO94/29444).

- Ensoli B.: HIV-1 Tat, or derivates thereof, alone or in combination, for prophylactic and therapeutic vaccination. (Italian patent, priority date December 1, 1997 N. 1297090; PCT November 30, 1998, No. PCT/EP98/07721, Publication No. WO99/27958; European Patent Application No. 98966601.1, priority date November 30, 1998; European Patent Application N. 05026908.3-2402 [divisional of EP98966601.1]).

- Ensoli B.: Employment of human immunodeficiency virus (HIV) protease inhibitors (HIV-PI) for utilizing them as drugs or realizing new anti-angiogenic, anti-tumor, anti-edemic, anti-inflammatory drugs, for the treatment of Kaposi's sarcoma, tumors and angioproliferative,

inflammatory and autoimmune diseases in HIV infected or non infected subjects (European Patent application, No. RM2001 A000210, priority date April 18, 2001; PCT April 18, 2002, No. PCT/EP02/04303, Publication No.WO02/087583).

- Ensoli B.: Use of biologically active HIV-1 Tat, fragments or derivatives thereof, to target and/or to activate antigen-presenting cells, and/or to deliver cargo molecules for preventive or therapeutic vaccination and/or to treat infectious diseases, inflammatory and angiogenic diseases, and tumors. (European Patent application, No. 01118114.6, priority date July 26, 2001, PCT July 26, 2002, No. PCT/EP02/08377, Publication No. WO03/009867).

- Ensoli B., Gavioli R., Caputo A.: Vaccines (United Kingdom Patent application, No. 0323840.9, priority date October 10, 2003; PCT October 10, 2004, No. PCT/EP2004/11950, Publication No. WO2005/039631).

- Ensoli B., Caputo A., Laus M., Tondelli L., Sparnacci K. Nanoparticles for delivery of a pharmacologically active agent (United Kingdom Patent application, No. 0325625.2, priority date November 3, 2003; PCT November 3, 2004, No. PCT/EP2004/012420, Publication No. WO2005/048997).

- Ensoli B., Caputo A., Gavioli R., Tondelli L., Laus M., Sparnacci K. Use of microparticles for antigen delivery (United Kingdom Patent application, No. 0325624.5, priority date November 3, 2003; PCT November 3, 2004, No. PCT/EP2004/012421, Publication No. WO2005/049093).

- Ensoli B. Novel Tat complexes, and vaccines comprising them (United Kingdom Patent application, No. 0405480.5, priority date March 11, 2004; PCT March 11, 2005, No. PCT/EP2005/003043, Publication No. WO2005/090391).

- Ensoli B., Peng B., Voltan R., Robert-Guroff M. Improved replication-competent adenoviral vectors (Provisional US Patent Application, No. 60/629,722 – filed on November 18, 2004; Patent application, No 11/282,319 filed on November 17, 2005, No. US2006/0115456).

Editorial Board Member:

Lancet Oncology

Reviewer for Journals:

AIDS
AIDS Research and Human Retroviruses
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Cancer
Cancer Research
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European Journal of Immunology

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FEBS Letters
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International Journal of Cancer
Journal of Acquired Immunodeficiency Syndrome
Journal of Clinical Investigation
Journal of General Virology
Journal of Immunology
Journal of Infectious Diseases
Journal of Neuroscience
Journal of Virology
Laboratory Investigation
Microvascular Research
Nature
Nature Medicine
New England Journal of Medicine
Oncogene
Proceedings of the National Academy of Sciences, USA
The American Journal of Pathology
Trends in Biotechnology
Vaccine
Virology

Reviewer of Grants for:

- Louisiana Education Quality Support Fund, Research and Development Program NIH Grants, USA
- NIH Grants, Ohio State University Comprehensive Cancer, The Ohio State University, USA
- Center National Health Research and Development Program, Canada
- Wellcome Trust registered Charity, London, UK
- Extramural AIDS grants, Ministry of Health, Italy
- European Molecular Organization (EMBO), Germany

**Experiences related to HIV/AIDS**

Study of virus-host interaction with particular regard to the effects of viral products. First demonstration that the Tat protein of HIV-1 is released by acutely infected cells and exerts key activities on the virus and on endothelial cells. Role of the HIV-1 Tat protein, cytokines and HHV-8 in the pathogenesis of AIDS-associated Kaposi's Sarcoma. Biology of angiogenic growth factors. Gene therapy approaches for the inhibition of HIV-1 replication *in vitro* and AIDS-associated tumor development *in vivo*. Role of HIV-1 protease inhibitors as anti-angiogenic factors in promoting regression of AIDS-Kaposi's sarcoma. Development of anti-HIV vaccines based on Tat protein or Tat-DNA which were found capable of inhibiting virus replication and AIDS development in monkeys. Studies on the role of Tat as adjuvant: uptake by dendritic cells, induction of their maturation and promotion of their capacity to present antigens and to elicit T cell responses to other antigens. Studies on the humoral and cellular immune response to virus antigens during the natural course of infection and correlation with disease stages. Standardization of tests for the evaluation of humoral and cellular immune response to viral proteins and virological parameters for monitoring immunogenicity and efficacy of vaccine candidates in clinical trials. New immunization strategies to induce both systemic and mucosal immunity. Studies of vaccination strategies based on the combination of Tat with other viral regulatory and structural proteins (Env). Cooperation with the industry to the development of novel therapeutic strategies. Implementation of production and purification protocols for large scale GLP and GMP production of candidate antigens. Organization and preparation of phase I preventive and therapeutic clinical trials for the Tat-based vaccine against HIV/AIDS in Italy; organization of phase II clinical trials in Italy and Africa (Uganda, South Africa and Swaziland).

**Field Experiences related to HIV/AIDS**

Role of Tat protein of HIV-1 and the Human Herpes virus 8 (HHV-8) in the pathogenesis of Kaposi's Sarcoma, an angioproliferative disease which is present at high frequencies in Africa. Studies on transmission of HHV-8 infection in Egypt, Cameroon, Uganda and Papua New Guinea, demonstrating non sexual transmission of HHV8 among adults and absence of vertical transmission (mother-to-child) in children. Membership of an International Collaborative Group and participation in studies on variability and evaluation of Kaposi's sarcoma-associated herpesvirus in Europe and Africa. Immunological and virological studies in the field of HIV infection in African countries (South Africa, Swaziland, Uganda), preliminary to phase II/III clinical trials with a vaccine against HIV/AIDS based on the biologically-active Tat protein, developed at ISS. Molecular epidemiological studies in Uganda and South Africa for determination of locally circulating HIV subtypes. Studies on variability of the HIV viral proteins in Uganda and South Africa in field isolates from patients infected with different HIV subtypes. Standardization of immunological and virological tests in genetically and environmentally different human populations in Africa (Uganda, South Africa, Swaziland) where phase II/III trials with the Tat-based vaccine are planned. Epidemiological feasibility studies to identify potential cohorts for phase II/III clinical trials. Studies in Uganda demonstrating the presence of an immune activation in HIV-infected African patients environmentally driven and associated with up-regulation of the HIV co-receptor CCR5. Establishment of collaborations with African countries for the development of clinical trials with the Tat-based vaccine. Membership of the WHO-UNAIDS vaccine advisory committee, vice-president of the Science Committee of UNESCO and delegate for Italy to the United Nations.

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2. Aiuti F., **Ensoli B.**, Scarpati B., Carbonari M., Scano G., Papetti C., Sirianni M.C., and Rossi P. Nuovi aspetti eziologici, epidemiologici ed immunologici della sindrome da immunodeficienza acquisita (AIDS) e delle sindromi ad essa correlate in Italia. Med. Riv. EMI (Italian) 4: 383-390, 1984.

3. Aiuti F., D'Amelio R., Paganelli R., Cherchi M., **Ensoli B.**, and Scano G. Intestinal pathology in immunodeficiencies and autoimmune diseases. In Vierucci, A. (Ed.): Immunity and Infections in the Intestinal Tract and Liver in Children. Florence, Masson Italia, 1984, pp. 5-59.

4. Paganelli R., Le Moli S., **Ensoli B.**, Seminara R., Cabello A., Gaetano G., Cherchi M., Venuta S., and Aiuti F. Production of interleukin-2 and receptor expression in primary and acquired immunodeficiencies. In 8th Conference of the Italian Society of Immunology and Immunopathology (Italian). Milan, O.I.C. Medical Press, 1984, pp. 73-77.

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6. Aiuti F., Paganelli R., **Ensoli B.**, Cabello A., Cherchi M., Venuta S., Capobianchi M.R., and Dianzani F. Abnormalities of T cell subsets and lymphokines (IL-2 and gamma IFN) in immunodeficiencies. In Aiuti, F., Rosen, F., and Cooper, M.D. (Eds.): Recent Advances in Primary and Acquired Immunodeficiencies. New York, Raven Press, 1984, pp. 155-164.

7. Crescenzi M., Carbonari M., Bonomo R., **Ensoli B.**, Soddu S., and Cafaro A. Clinical use with gamma globulin IV (endobulin) Quaderni di Medicina e Chirurgia (Italian) 8: 151-153, 1984.

8. Fiorilli M., Quinti I., Russi G., Seminara R., **Ensoli B.**, and Aiuti F. Thymopentin treatment for selective IgA deficiency. Karger's Surv. Immunol. Res. 4 (Suppl. 1): 129-134, 1985.

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10. Aiuti F., Capobianchi M.R., Paganelli R., **Ensoli B.**, Cabello A., Sirianni M.C., Facchini J., and Dianzani F. Further studies on interferon-gamma production in patients with primary immunodeficiency. In The Interferon System. New York, Raven Press, 1985, pp. 397-403.

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12. Pandolfi F., De Rossi G., Mariani G., Carbonari M., **Ensoli B.**, Napolitano M., Lopez M., Mandelli F., and Aiuti F. Impairment of cellular immunity and OKT4 lymphocytes in symptom-

free hemophiliacs with antibodies to human T leukemia virus III (HTLV III). Diagnostic Immunol. 3: 155-159, 1985.

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19. Businco L., Rubaltelli F.F., Paganelli R., Galli E., **Ensoli B.**, Betti P., and Aiuti F. Results in two infants with Digeorge syndrome-Effects of long-term TP5. Clin. Immunol. Immunopathol. 39: 222-230, 1986.

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syndrome": A form of severe combined immunodeficiency with phenotypically normal T cells, elevated IgE, and eosinophilia. Clin. Immunol. Immunopathol. 44: 123-133, 1987.

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Ufficio Stampa
*Viale Regina Elena, 299*
*00161 Roma*

Tel. +39 06 4990.2950
*Fax +39 06 4938.7154*
e-mail: ufficio.stampa@iss.it
www.iss.it

## COMUNICATO STAMPA N° 06/05

# The HIV TAT vaccine meets the goals of the phase I trials. Results released by the Italian National Institute of Health

Today the Italian National Institute of Health (Istituto Superiore di Sanità, ISS) has released the 24 weeks interim results of both preventive and therapeutic phase I trials with the TAT vaccine, developed by Dr. Barbara Ensoli's team.

Both trials are randomized, double blinded and placebo controlled. The vaccine is composed of the recombinant active TAT protein of HIV - a key regulatory protein, which is essential for the virus replication. The vaccine is aimed at controlling the virus growth and blocking the disease onset.

Five immunizations of three different doses of the vaccine or the placebo were given subcute or intradermally to 20 healthy individuals at no risk of infection and 27 HIV+ clinically asymptomatic subjects not on antiretroviral therapy.

**The vaccine has completely met both primary and secondary endpoints:** it has proved to be safe and well tolerated in all subjects and **very encouraging results of immunogenicity have been obtained.**

**100%** of the vaccinated volunteers developed specific antibodies and **more than 80%** developed cellular immune responses in both protocols.

The two trials sponsored by ISS and coordinated by Dr. Barbara Ensoli have been conducted in parallel in four Italian clinical centers: San Raffaele Hospital in Milan, San Gallicano Hospital, Lazzaro Spallanzani Hospital and Policlinico Umberto I in Rome.

The results encourage the Italian vaccine program to proceed towards phase II studies both in Italy and in developed countries. This will allow to test the vaccine in those populations (i.e. high risk individuals and HIV infected people on treatment) that will represent the target to prove the efficacy in phase III trials.

Rome, the 5-th of July 2005

**Table 1**

***ISS P-001* - Frequency of anti-Tat humoral responses**

| | IgM | IgG | IgA |
|---|---|---|---|
| **TAT+ALUM, SC** | | | |
| **7.5** | 3/3 (100%) | 3/3 (100%) | 3/3 (100%) |
| **15** | 3/3 (100%) | 3/3 (100%) | 3/3 (100%) |
| **30** | 2/2 (100%) | 2/2 (100%) | 2/2 (100%) |
| **TOTAL** | **8/8 (100%)** | **8/8 (100%)** | **8/8 (100%)** |
| | | | |
| **TAT, ID** | | | |
| **7.5** | 2/2 (100%) | 2/2 (100%) | 1/2 (50%) |
| **15** | 2/2 (100%) | 2/2 (100%) | 1/2 (50%) |
| **30** | 2/2 (100%) | 2/2 (100%) | 2/2 (100%) |
| **TOTAL** | **6/6 (100%)** | **6/6 (100%)** | **4/6 (67%)** |
| | | | |
| **TOTAL VACCINEES** | **14/14 (100%)** | **14/14 (100%)** | **12/14 (86%)** |
| | | | |
| **PLACEBO SC** | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
| **PLACEBO ID** | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) |
| **TOTAL PLACEBO** | **0/4 (0%)** | **0/4 (0%)** | **0/4 (0%)** |

**Fig. 1**

***ISS P-001*** **– Anti-Tat IgM, IgG and IgA by dosage group and route of administration (average titers) (■ SC □ ID)**

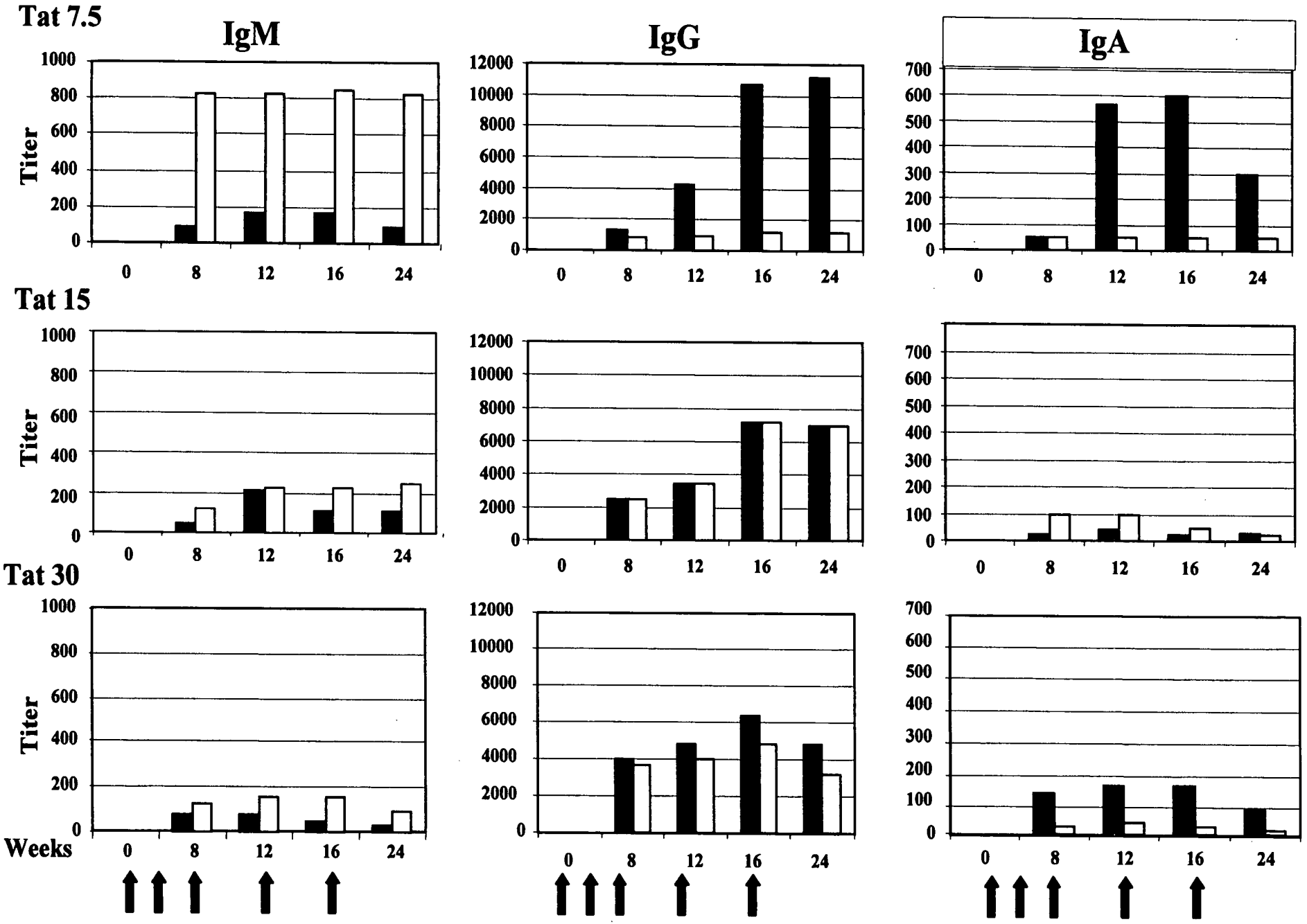


Fig. 2

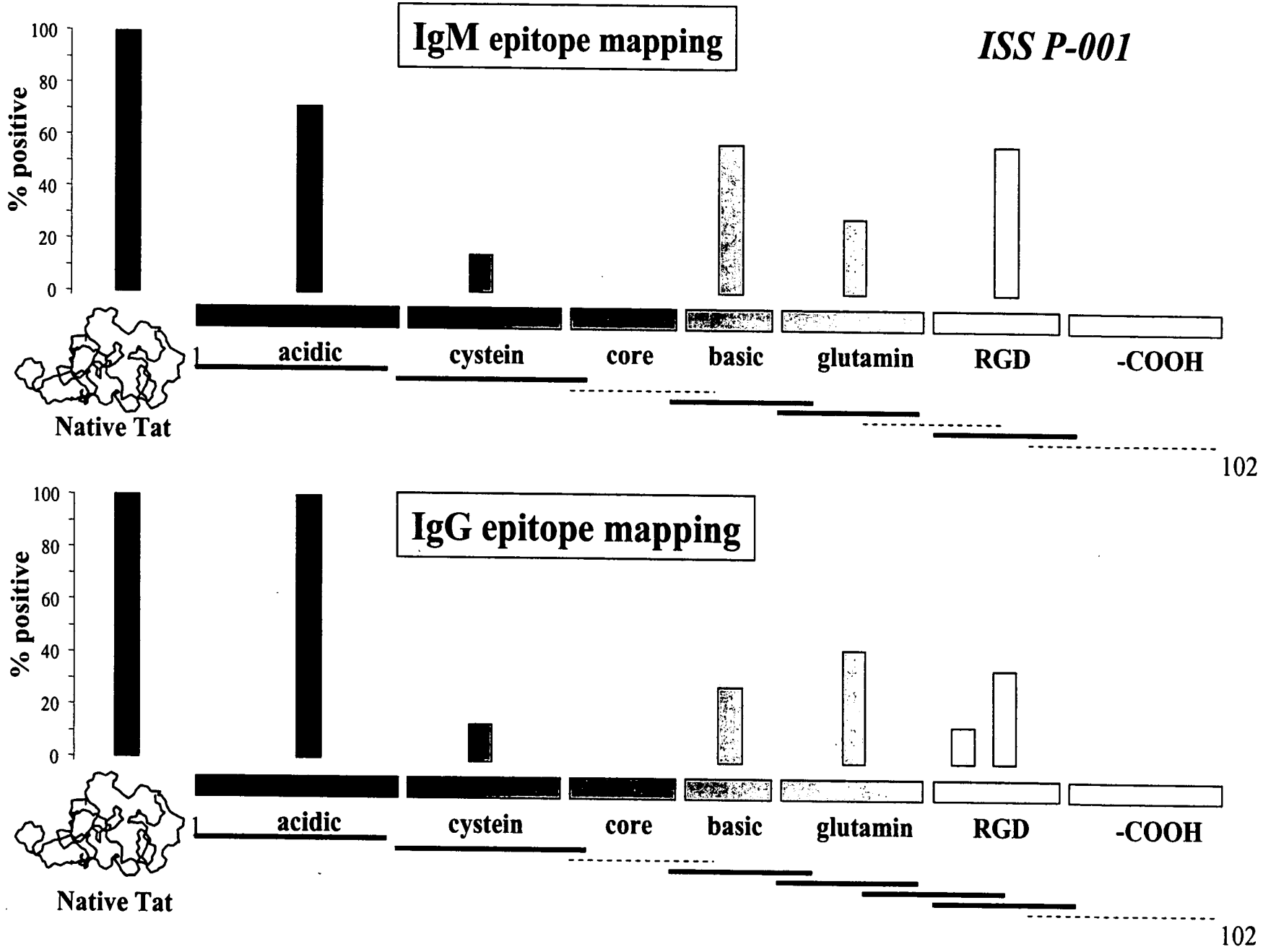

IgM epitope mapping
ISS P-001
% positive
100
80
60
40
20
0
acidic
cystein
core
basic
glutamin
RGD
-COOH
Native Tat
1
102
IgG epitope mapping
% positive
100
80
60
40
20
0
acidic
cystein
core
basic
glutamin
RGD
-COOH
Native Tat
1
102

**Fig. 3**

***ISS P-001* –50% neutralizing anti-Tat antibody titers in vaccinees by dosage and route (■ SC □ ID)**

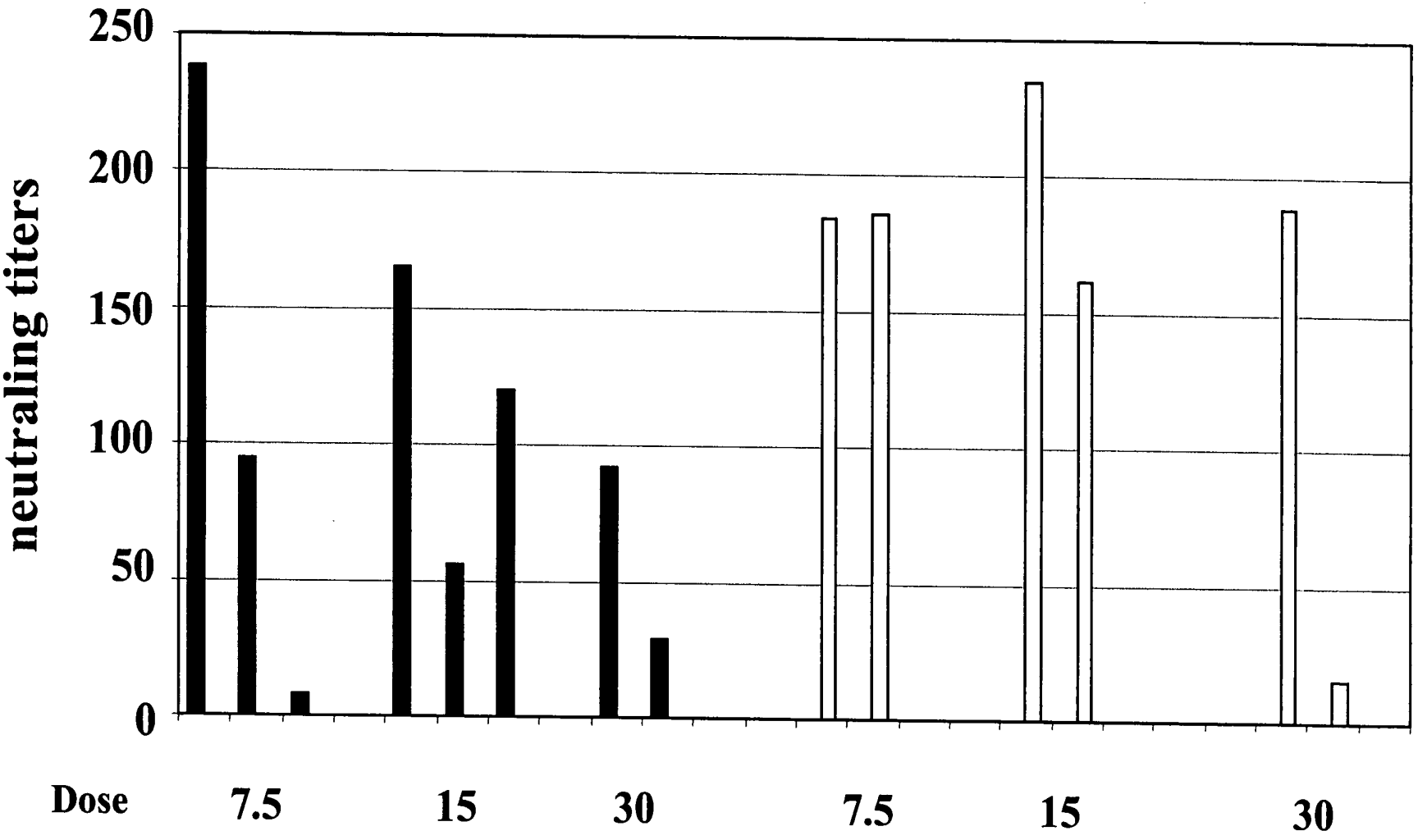
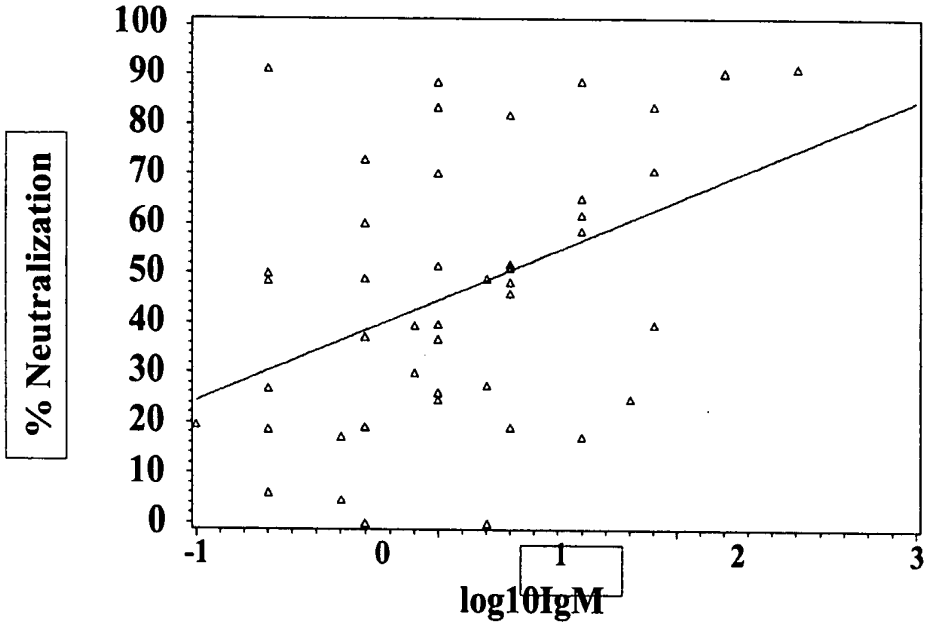
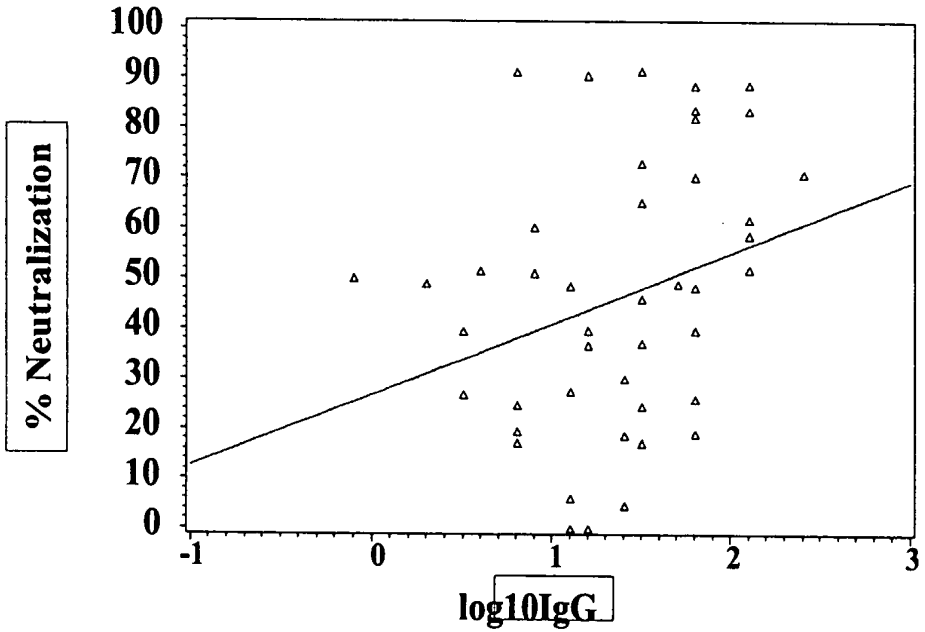


**Fig. 4**

***ISS P-001* – Correlation of Anti-Tat binding IgM and IgG Antibodies with neutralizing activity**




**IgM vs Neutralization:**
**r=0.4 p=0.0039**




**IgG vs Neutralization:**
**r=0.3 p=0.0541**

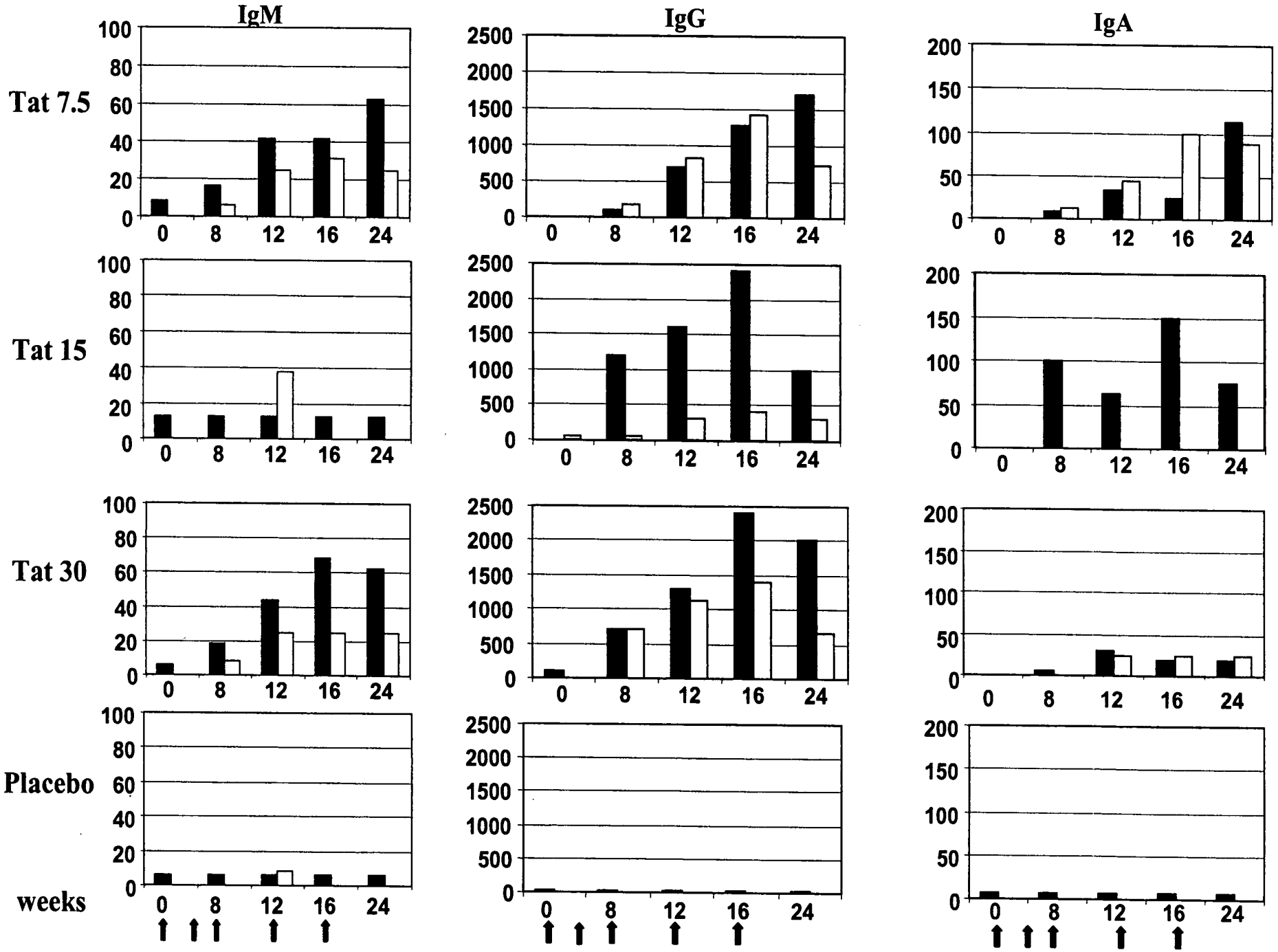
## Table 2

## *ISS T-001* - Frequency of anti-Tat humoral responses

| | IgM | | IgG | | IgA | |
|---|---|---|---|---|---|---|
| | **Before** | **After** | **Before** | **After** | **Before** | **After** |
| **Tat + Alum, SC** | | | | | | |
| **7.5 µg** | 1/3 (33%) | 2/3 (67%) | 0/3 (0%) | 3/3 (100%) | 0/3 (0%) | 2/3 (67%) |
| **15 µg** | 1/2 (50%) | 1/2 (50%) | 0/2 (0%) | 2/2 (100%) | 0/2 (0%) | 2/2 (100%) |
| **30 µg** | 1/4 (25%) | 4/4 (100%) | 1/4 (25%) | 4/4 (100%) | 0/4 (0%) | 2/4 (50%) |
| **Total** | **3/9 (33%)** | **7/9 (78%)** | **1/9 (11%)** | **9/9 (100%)** | **0/9 (0%)** | **6/9 (67%)** |
| **Tat, ID** | | | | | | |
| **7.5 µg** | 0/4 (0%) | 4/4 (100%) | 0/4 (0%) | 4/4 (100%) | 0/4 (0%) | 3/4 (75%) |
| **15 µg** | 0/2 (0%) | 2/2 (100%) | 1/2 (50%) | 2/2 (100%) | 0/2 (0%) | 0/2 (0%) |
| **30 µg** | 0/3 (0%) | 2/3 (67%) | 0/3 (0%) | 3/3 (100%) | 0/3 (0%) | 2/3 (67%) |
| **Total** | **0/9 (0%)** | **8/9 (88%)** | **1/9 (11%)** | **9/9 (100%)** | **0/9 (0%)** | **5/9 (55%)** |
| | | | | | | |
| **TOTAL VACCINEES** | **3/18 (17%)** | **15/18 (83%)** | **2/18 (11%)** | **18/18 (100%)** | **0/18 (0%)** | **11/18 (61%)** |
| | | | | | | |
| **Placebo, SC** | 1/4 (25%) | 1/4 (25%) | 1/4 (25%) | 1/4 (25%) | 1/4 (25%) | 1/4 (25%) |
| **Placebo, ID** | 0/3 (0%) | 1/3 (33%) | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) |
| **TOTAL PLACEBO** | **1/7 (14%)** | **2/7 (29%)** | **1/7 (14%)** | **1/7 (14%)** | **1/7 (14%)** | **1/7 (14%)** |

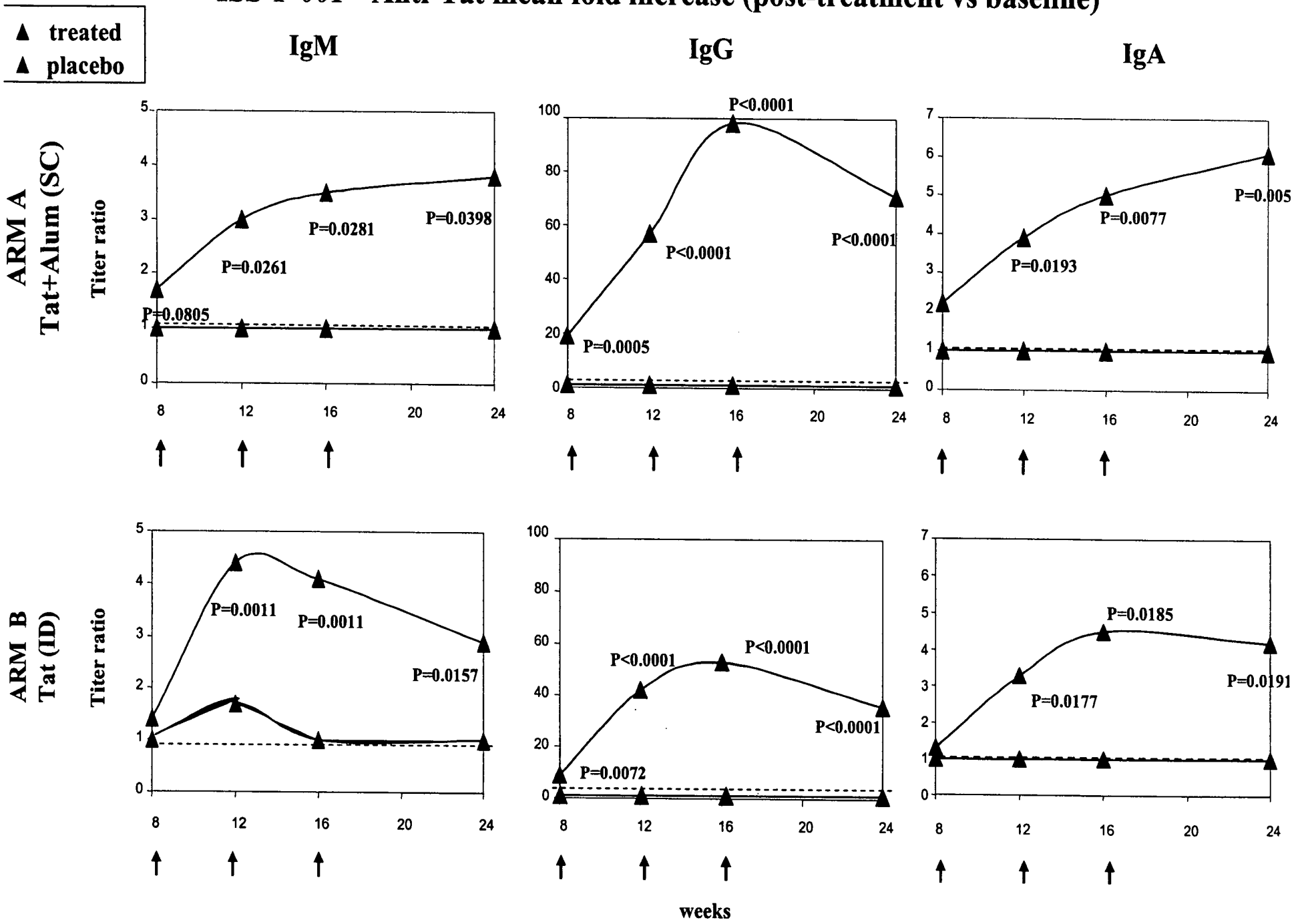
**Fig. 5**

***ISS T-001*** **- Anti-Tat IgM, IgG and IgA (Average titers) by dosage and route (■SC and□ID)**

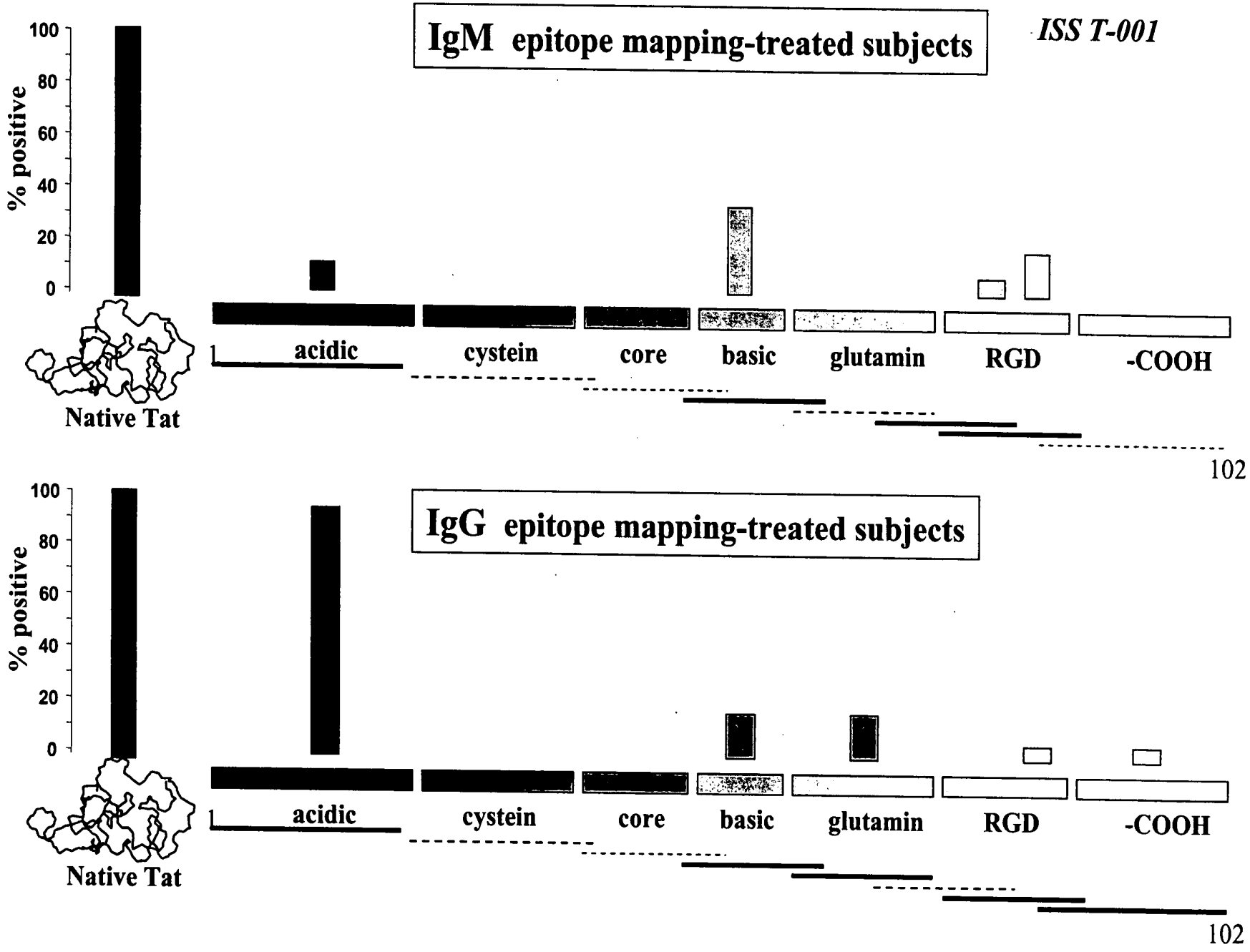


# Fig. 6

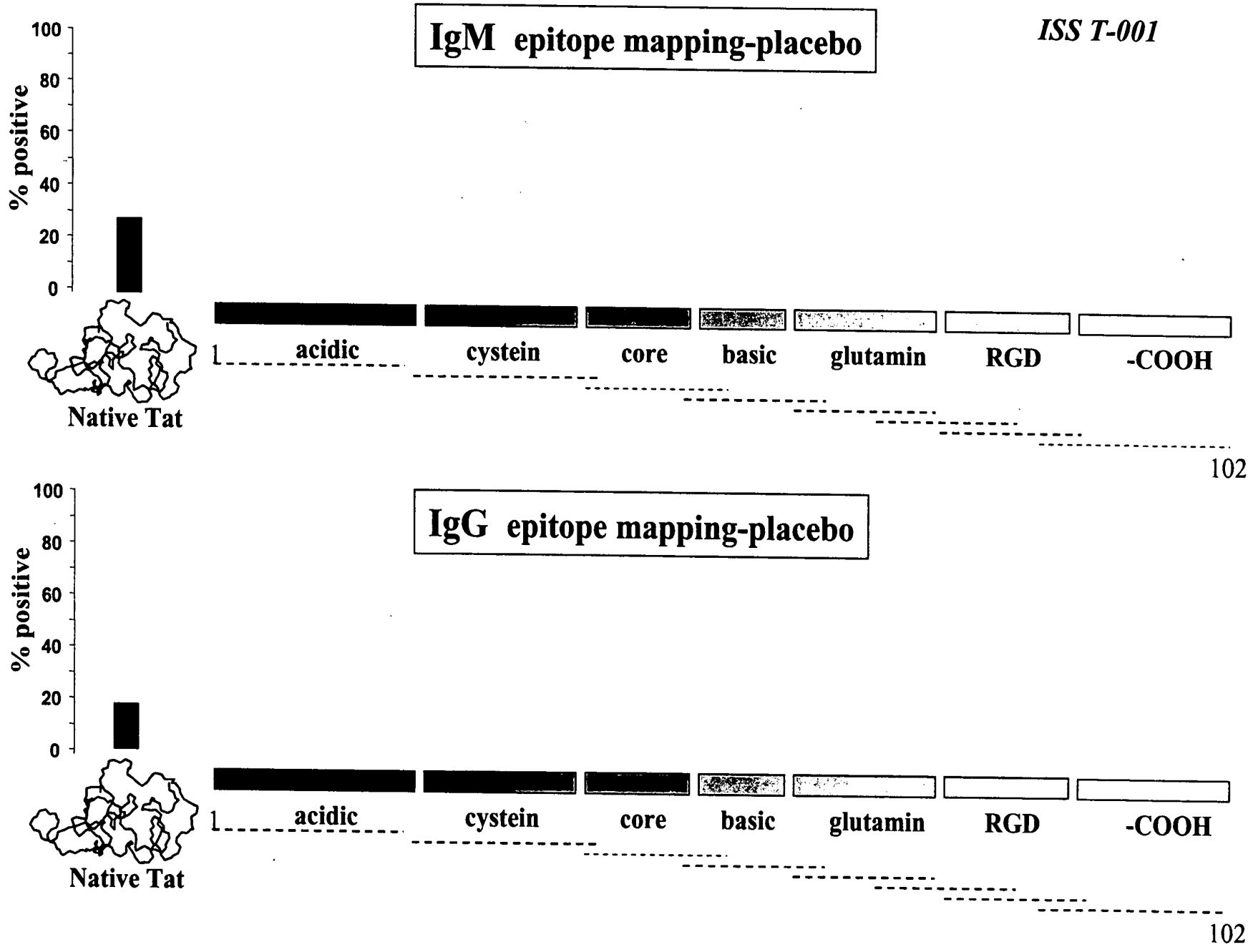
## *ISS T-001* - Anti-Tat mean fold increase (post-treatment vs baseline)



# Fig. 7


IgM epitope mapping-treated subjects
ISS T-001
% positive
100
80
60
40
20
0
acidic
cystein
core
basic
glutamin
RGD
-COOH
Native Tat
102
IgG epitope mapping-treated subjects
% positive
100
80
60
40
20
0
acidic
cystein
core
basic
glutamin
RGD
-COOH
Native Tat
102

# Fig. 8


IgM epitope mapping-placebo
ISS T-001
% positive
100
80
60
40
20
0
Native Tat
1
acidic
cystein
core
basic
glutamin
RGD
-COOH
102
IgG epitope mapping-placebo
% positive
100
80
60
40
20
0
Native Tat
1
acidic
cystein
core
basic
glutamin
RGD
-COOH
102

# Fig. 9

## *ISS T-001* - Anti-Tat Neutralization Activity by dosage and Arm

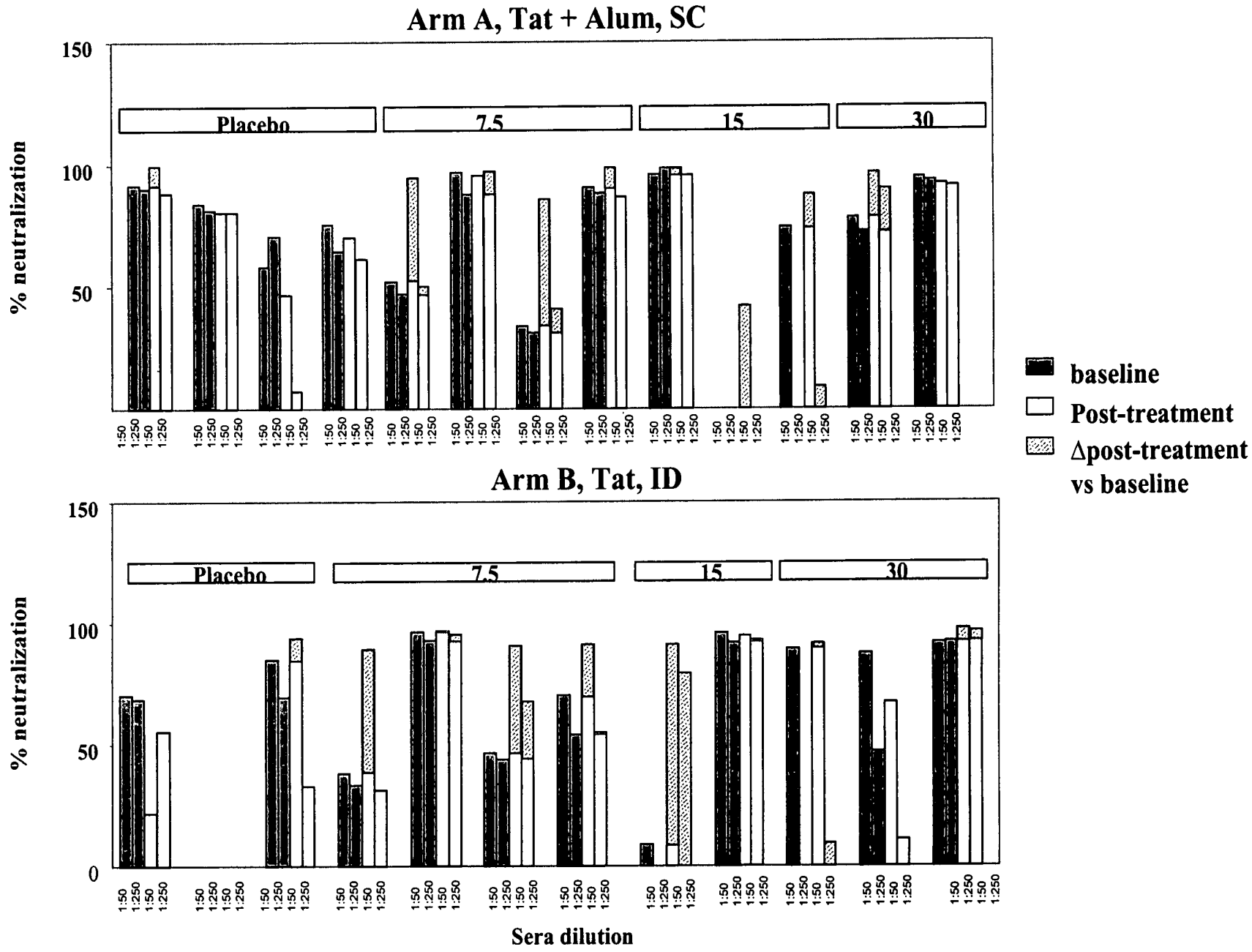


**Fig. 10**

***ISS T-001*** **– Ratio of anti-Tat IgM or IgG binding antibodies versus baseline, and Δ of neutralization versus baseline (after III immunization)**

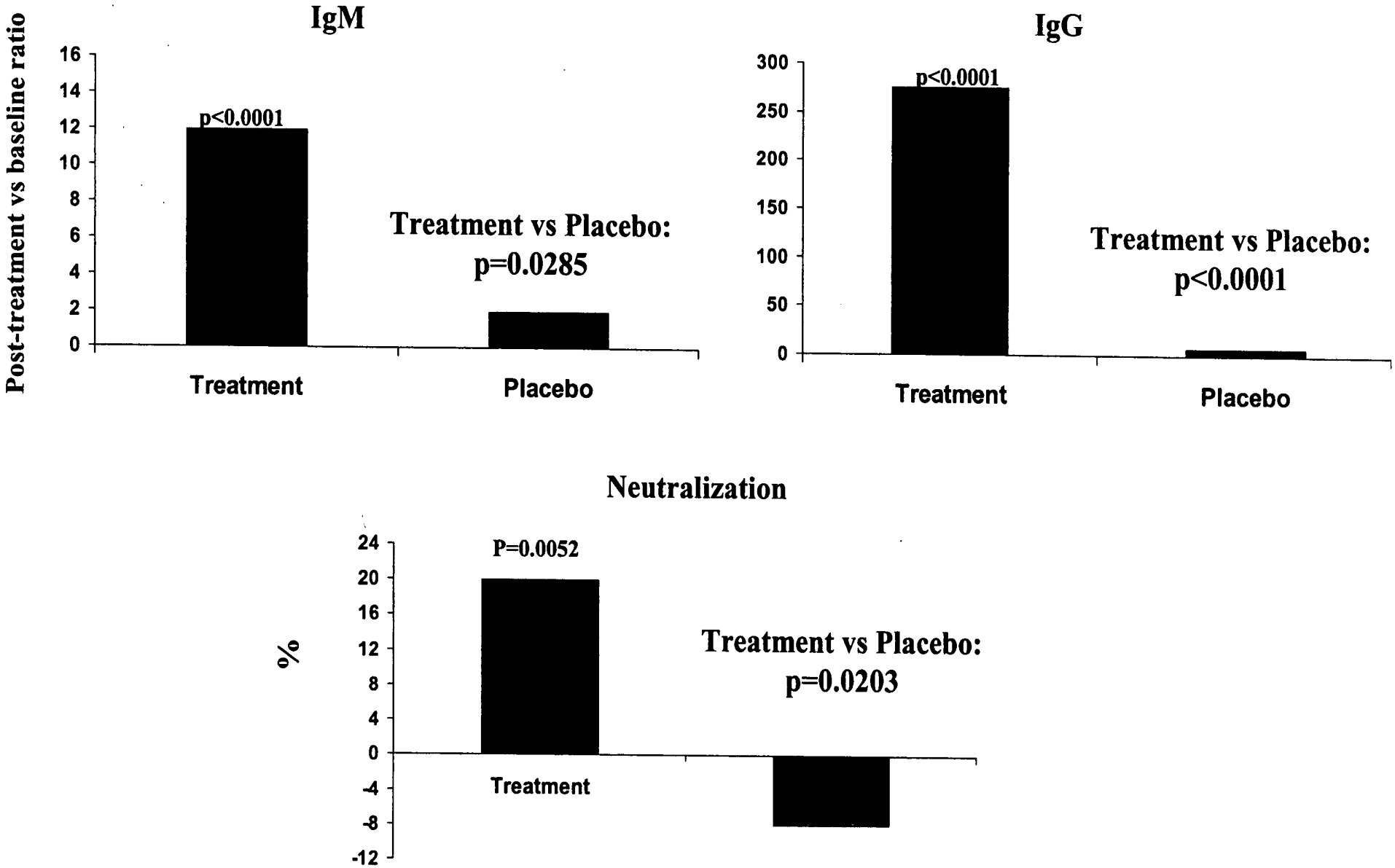


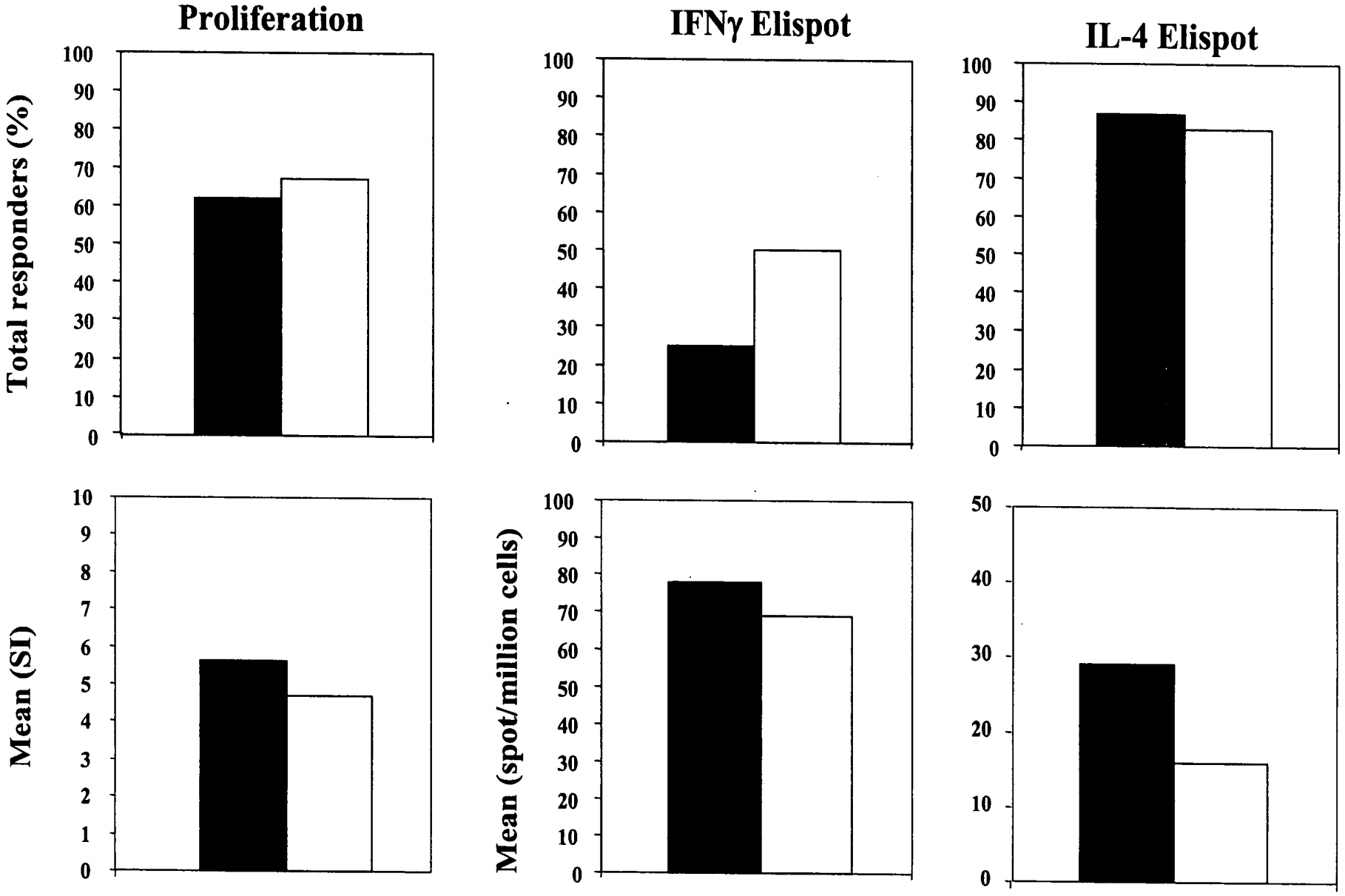
**Table 3**

***ISS P-001* - Frequency of anti-Tat cellular responses**

| | CUMULATIVE RESPONSE | Proliferation | IFN γ Elispot | IL4 Elispot |
|---|---|---|---|---|
| **TAT+ALUM, SC** | | | | |
| **7.5 μg** | 3/3 (100%) | 2/3 (67%) | 0/3 (0%) | 3/3 (100%) |
| **15 μg** | 2/3 (67%) | 1/3 (33%) | 1/3 (33%) | 2/3 (67%) |
| **30 μg** | 2/2 (100%) | 2/2 (100%) | 1/2 (50%) | 2/2 (100%) |
| **TOTAL** | **7/8 (87%)** | **5/8 (62.5%)** | **2/8 (25%)** | **7/8 (87.5%)** |
| | | | | |
| **TAT, ID** | | | | |
| **7.5 μg** | 2/2 (100%) | 1/2 (50%) | 1/2 (50%) | 1/2 (50%) |
| **15 μg** | 2/2 (100%) | 2/2 (100%) | 0/2 (0%) | 2/2 (100%) |
| **30 μg** | 2/2 (100%) | 1/2 (50%) | 2/2 (100%) | 2/2 (100%) |
| **TOTAL** | **6/6 (100%)** | **4/6 (67%)** | **3/6 (50%)** | **5/6 (83%)** |
| | | | | |
| **TOTAL VACCINEES** | **13/14 (93%)** | **9/14 (64%)** | **5/14 (36%)** | **12/14 (86%)** |
| | | | | |
| **PLACEBO, SC** | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
| **PLACEBO, ID** | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) | 0/3 (0%) |
| **TOTAL PLACEBO** | **0/4 (0%)** | **0/4 (0%)** | **0/4 (0%)** | **0/4 (0%)** |

Fig. 11

*ISS P-001* – Cellular responses: frequency of total responders and mean values of intensity by route (■SC □ID)



# Table 4

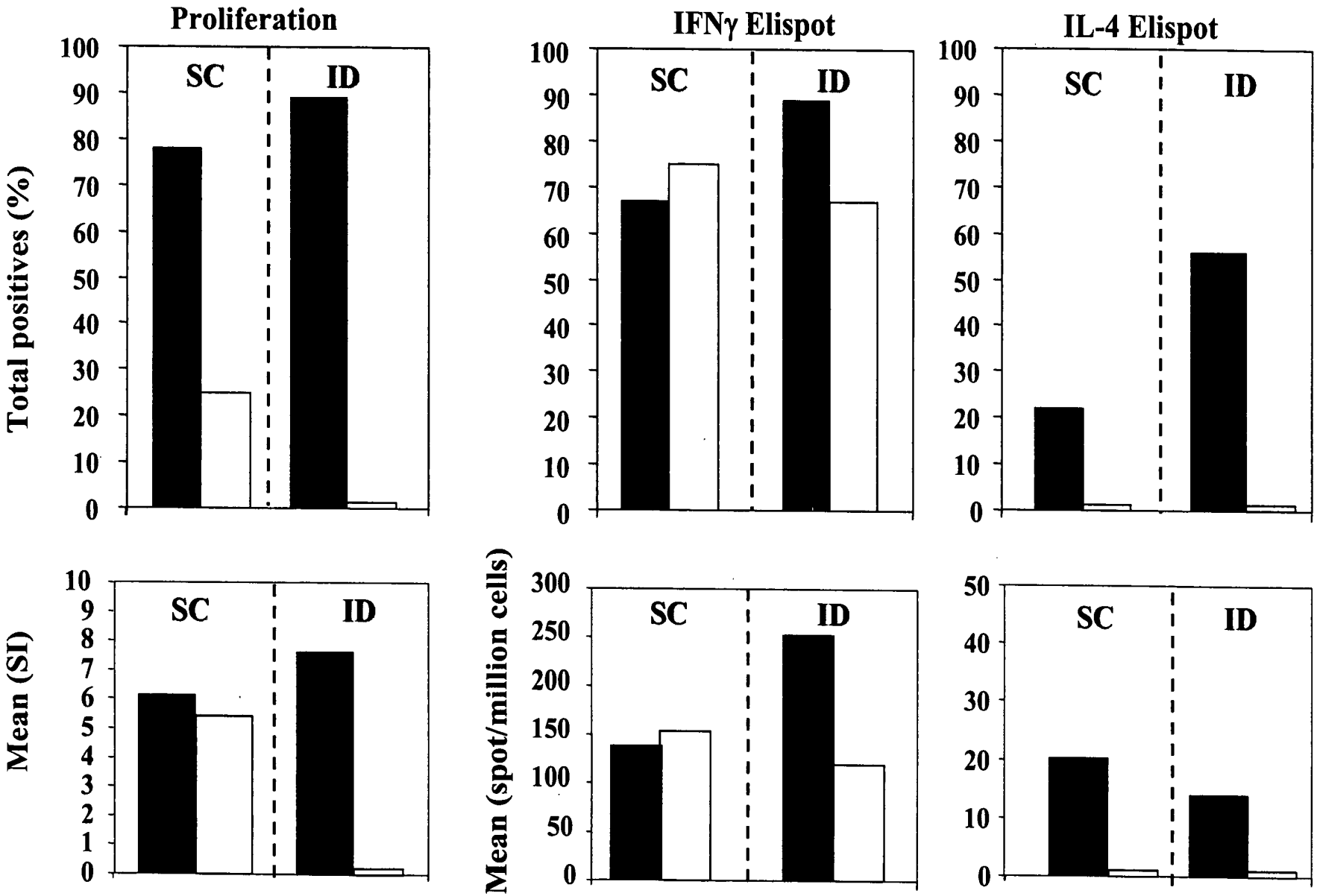
## *ISS T-001* - Frequency of anti-Tat cellular responses prior to (baseline) or after vaccination

| | Total Response | | Proliferation | | IFNγ Elispot | | IL 4 Elispot | |
|---|---|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After | Before | After |
| Tat+Alum, SC | | | | | | | | |
| 7.5 | 2/3 (67%) | 3/3 (100%) | 1/3 (33%) | 2/3 (67%) | 1/3 (33%) | 1/3 (33%) | 1/3 (33%) | 0/3 (0%) |
| 15 | 2/2 (100%) | 2/2 (100%) | 1/2 (50%) | 2/2 (100%) | 1/2 (50%) | 1/2 (50%) | 0/2 (0%) | 1/2 (50%) |
| 30 | 4/4 (100%) | 4/4 (100%) | 4/4 (100%) | 3/4 (75%) | 2/4 (50%) | 4/4 (100%) | 0/4 (0%) | 1/4 (25%) |
| **Total** | **8/9 (89%)** | **9/9 (100%)** | **6/9 (67%)** | **7/9 (78%)** | **4/9 (44%)** | **6/9 (67%)** | **1/9 (11%)** | **2/9 (22%)** |
| Tat, ID | | | | | | | | |
| 7.5 | 3/4 (75%) | 4/4 (100%) | 2/4 (50%) | 3/4 (75%) | 2/4 (50%) | 3/4 (75%) | 1/4 (25%) | 3/4 (75%) |
| 15 | 1/2 (50%) | 2/2 (100%) | 1/2 (50%) | 2/2 (100%) | 1/2 (50%) | 2/2 (100%) | 1/2 (50%) | 0/2 (0%) |
| 30 | 3/3 (100%) | 3/3 (100%) | 2/3 (67%) | 3/3 (100%) | 3/3 (100%) | 3/3 (100%) | 0/3 (0%) | 2/3 (67%) |
| **Total** | **7/9 (78%)** | **9/9 (100%)** | **5/9 (55%)** | **8/9 (89%)** | **6/9 (67%)** | **8/9 (89%)** | **2/9 (22%)** | **5/9 (55%)** |
| | | | | | | | | |
| **TOTAL VACCINEES** | **15/18 (83%)** | **18/18 (100%)** | **11/18 (61%)** | **15/18 (83%)** | **10/18 (55%)** | **14/18 (78%)** | **3/18 (17%)** | **7/18 (39%)** |
| | | | | | | | | |
| Placebo, SC | 4/4 (100%) | 3/4 (75%) | 1/4 (25%) | 1/4 (25%) | 3/4 (75%) | 3/4 (75%) | 1/4 (25%) | 0/4 (0%) |
| Placebo, ID | 2/3 (67%) | 1/3 (33%) | 1/3 (33%) | 0/3 (0%) | 2/3 (67%) | 1/3 (33%) | 0/3 (0%) | 0/3 (0%) |
| **TOTAL PLACEBO** | **6/7 (86%)** | **4/7 (57%)** | **2/7 (29%)** | **1/7 (14%)** | **5/7 (71%)** | **4/7 (57%)** | **1/7 (14%)** | **0/7 (0%)** |

**Fig. 12**

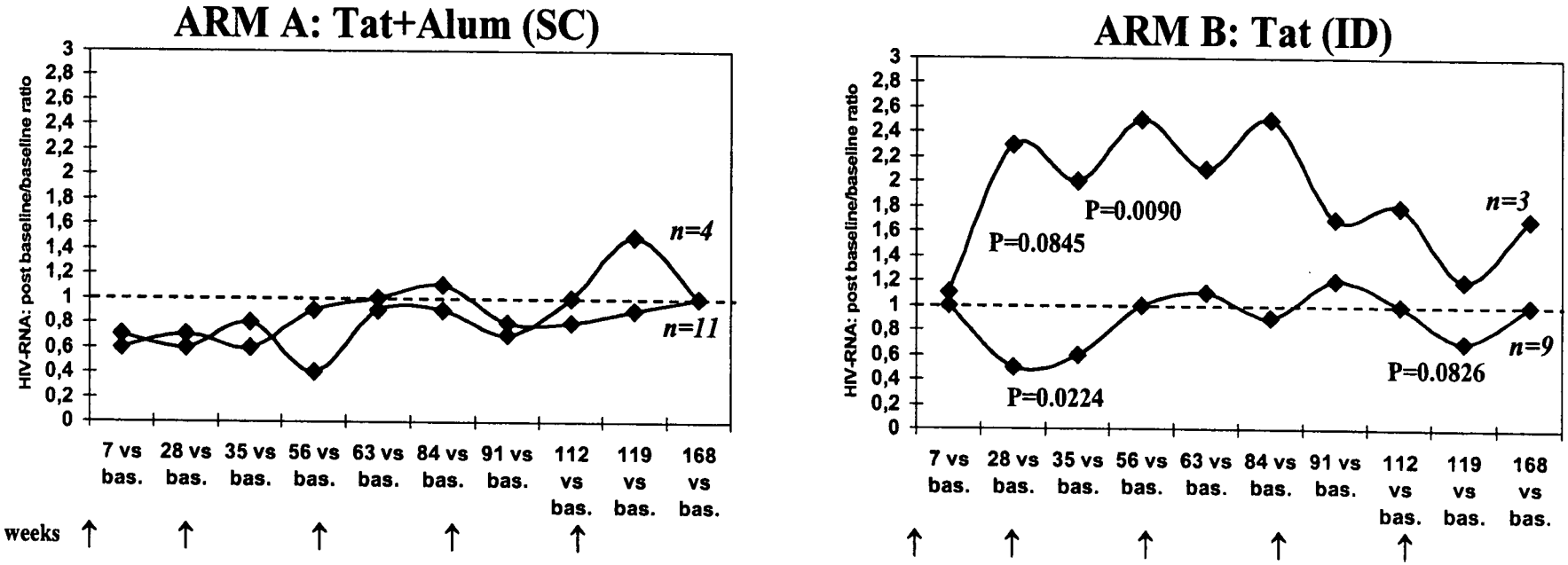
***ISS T001*** **– Cellular responses: total positive individuals and mean values by route**

**(■ Treated □ Placebo)**



# Fig. 13

## *ISS T-001* – Plasmaviremia:ratio post-treatment vs baseline



# Fig. 14

## *ISS T-001* - CD4+ T cells: D post-treatment vs baseline